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Global excellence in pathology: Africa

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Global excellence in pathology: Africa

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Table of contents

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PERSPECTIVE
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The Influence of Social Media in Promoting Knowledge Acquisition and Pathology Excellence in Nigeria

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The use of social media has evolved from platforms designed primarily for social connection and news sharing to include vibrant virtual academic environments. These platforms allow pathologists from across the globe to interact, exchange knowledge, and collaborate. Pathology in Nigeria, as in much of Africa, faces severe knowledge and practice gaps, with a lack of supporting modern laboratory infrastructure. Social media represents a potentially highly valuable avenue to help address some of these deficiencies. In this Perspective piece, we highlight our experience with the increasing role of social media in providing quality medical education in pathology globally, with an emphasis on how it bridges many of these gaps in Nigeria. Social media sites serve as sources of readily accessible, free, high-quality information to pathologists and trainees through academic discussions, quizzes, journal clubs, and informal consultations. They also provide opportunities for professional networking and research collaborations. Despite the availability and wide reach of these platforms, social media as a tool for advancement of knowledge in pathology is still undersubscribed in this part of the world. Improving awareness of and support for these tools will ideally help mitigate some of the challenges of practicing pathology in low and middle-income settings.

Keywords: social media, pathology, medical education, professional development, Nigeria, Africa

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INTRODUCTION

Beyond facilitating connections between individuals, social media use now significantly impacts political, commercial, and scientific circles. People of different ages, backgrounds, and geography use social media for a range of purposes within medicine. Pathologists engage in rich virtual academic environments that foster interaction, knowledge sharing, and collaboration, while not being limited by typical geographic, social, or cultural boundaries (1). An advantage of social media is the opportunity to curate information based on topics/areas of interest (2).

A decade ago, it may have been difficult to imagine social media as a powerful tool in medicine given the ethical and legal concerns surrounding patient privacy (3, 4). Many of these issues have now been addressed by published articles that provide guidelines for the professional use of social media by physicians, improving the adoptability of social media as a legitimate channel for discussing medical cases (1, 5–7). These sites have become hubs for a growing and enthusiastic worldwide audience to disseminate medical knowledge and foster professional networking (8).

The training and practice of pathology in many low- and middle-income countries, including Nigeria and much of Africa, is many decades behind what is currently obtainable in high-income countries. Social media can be a valuable tool to help ameliorate some of these deficiencies. Herein, we present our perspectives on the current role social media plays in providing a high-quality adjunct to formal medical education in Nigeria, contextualized within current social media use in pathology. Further, we consider relevant limitations and potential future directions.

PATHOLOGY PRACTICE IN AFRICA

Pathology services in most of Africa today lag considerably behind practice in high-income countries. This is principally due to limited resources with resultant poor healthcare financing (9). The budgetary allocation to the health sector in Nigeria for 2022 is almost 30% higher than it was for the previous year but still <5% of the total budget. This is far from the commitment made by leaders of the African Union in 2001 to allocate at least 15% of their annual budget to improve healthcare in Africa (10). Practicing in an environment that lacks requisite infrastructure greatly impairs routine services and the training of laboratory professionals, and it almost totally excludes the ability to conduct meaningful research. These inadequacies have severely impeded the fight against cancer (11). With prevailing low income and limited health insurance coverage, only a small proportion of the population can afford healthcare. As a result, most pathology laboratories process few samples, with the majority receiving <3,000 tissue samples annually (12). Low case volume and limited availability of ancillary techniques severely impair the proficiency of pathologists in this region. Indeed, in Nigeria, like nearly all of Africa, there is no current capacity for subspecialty training. Most training institutions do not have subscription access to journals or a repository of current literature. To illustrate, in one of the author's experience (O.O.F.), it is a struggle to get access to at least seven in 10 journals of interest. All these, coupled with low numbers of laboratory professionals have made pathologists almost invisible in this part of the world (12, 13).

PATHOLOGY AND SOCIAL MEDIA

The most commonly utilized social media platforms in pathology include Facebook, YouTube, Instagram, and Twitter. For an excellent review summarizing the use of these platforms in pathology, authors would refer interested readers to a recent article by Deeken et al. (14). In brief, Facebook has the most robust, global network and reach. Some pathologists share educational material in open professional accounts. Facebook groups allow interested parties to unite over a particular topic, even connecting pathologists and patients, and crowdsourcing images of rare entities for textbooks. Facebook allows posting of educational videos and, in particular, Facebook Live allows one to host interactive microscopy sessions. YouTube focuses on video sharing, widely accessible lectures, and case discussions, and it is the most popular site for these purposes. This platform facilitates access to the types of educational materials that are commonly sold for profit or Continuing Medical Education credits. It allows for a very wide distribution of materials, and videos may garner 50,000–100,000 views in one to several years. Instagram, a platform where images are central, is well-suited to pathology for its visual nature but is suboptimal for fostering conversations. Unlike other sites, one cannot share links or articles, impeding robust discussion (14). Twitter is a US-based microblogging site notable for its wide reach, visual nature, "open" network, and ability to encourage discourse around educational cases through creating and sharing "threads," where relevant information is embedded and linked. The ability to crowdsource cases and develop research collaborations are also strengths of Twitter (15). For the authors, Twitter is the platform with which we have the most professional experience, and we believe it to be uniquely suited to helping promote knowledge acquisition and pathology excellence. Therefore, we will largely focus on this platform for the remainder of this piece, while recognizing many of the elements are also applicable to other social media platforms.

Launched as "Twttr" in March 2007 as a free instant messaging service, today Twitter is a popular source of information for a wide variety of industries (16). In the last few years, a large number of pathology Twitter accounts have proliferated, including accounts for individuals, institutions and organizations/associations. In 2019, a list kept by Jerad Gardner, et al. (1) documented more than 4,700 active pathology-related Twitter accounts. Two years later, this number almost doubled (17). Pathology-related posts can be readily searched for by using the hashtag #PathTwitter, with many other specific tags for organ systems or subspecialties (e.g., #Dermpath for Dermatopathology and #GIpath for Gastrointestinal Pathology) or branches of pathology (e.g., #Cytopath for Cytopathology and #ForensicPath for Forensic Pathology) (7, 14, 18). See **Appendix** for **Supplementary Information**.

THE IMPACT OF TWITTER ON PATHOLOGY PRACTICE IN NIGERIA: OUR PERSPECTIVE

Very few studies have been done to assess the extent to which African pathologists use social media professionally (19–21). Many pathologists in this part of the world, as elsewhere, began using social media accounts primarily for personal purposes, most commonly WhatsApp and Facebook (**Figure 1**). Over the years, these platforms have increasingly been used to communicate with colleagues in the same region and sometimes seek consultations by sending images of difficult cases, although with limited use (20).

There is no official data on the exact number of pathologists practicing in Nigeria. About 2 years ago, the College of Nigerian Pathologists estimated that there were about 500 pathologists practicing in the country, a nation of over 200 million people (22). Most of these pathologists have no active presence on Twitter. Occasionally, a few pathology residents and consultants can be spotted engaging, rebroadcasting (retweeting) and commenting on pathology-related posts. Very few maintain active participation, posting pathology images to teach or to seek

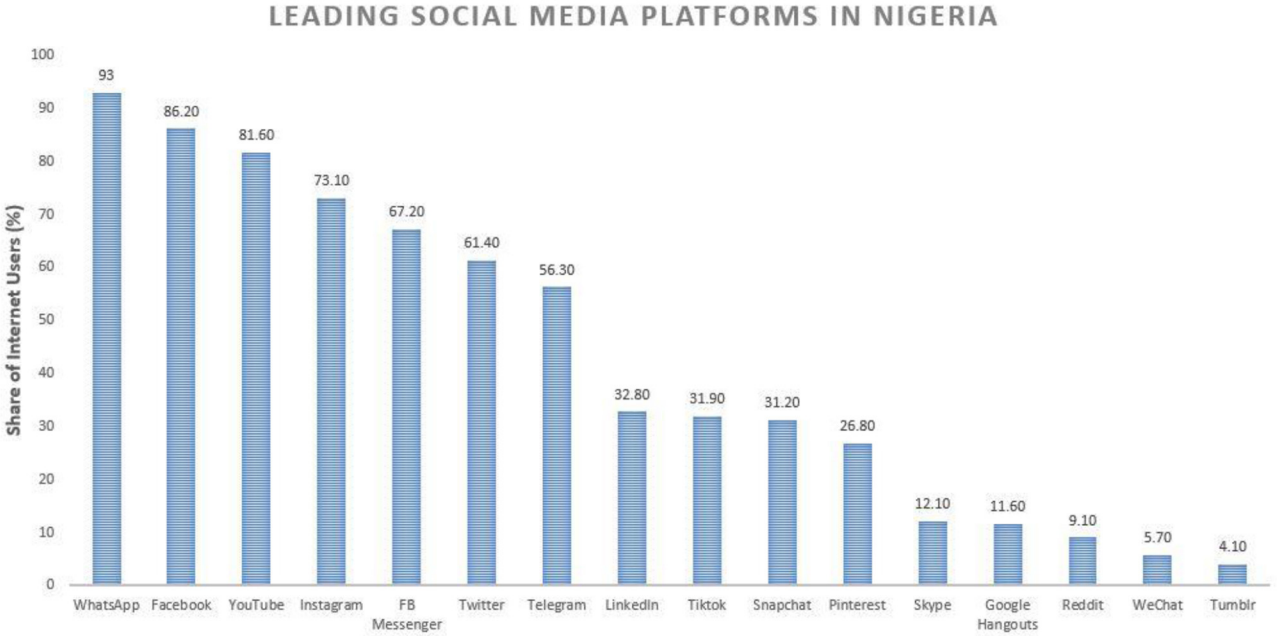

FIGURE 1 | A graph highlighting the popularity of various social media apps/sites commonly used in Nigeria. The information is based on survey data from internet users aged 16–64 years, as of the third quarter of 2020 (https://www.statista.com/statistics/1176101/leading-social-media-platforms-nigeria/) .

opinions in difficult cases. Therefore, the following discussion is based on the experience of these few, including two of the authors (O.O.F. and O.A.O).

In Nigeria, Twitter has been a force of positive impact in our pathology practice by providing: (1) a rich source of medical education; (2) a platform for case consultation; (3) a niche for professional networking and mentorship; and (4) a tool for research collaboration.

Twitter as a Source of High-Quality, Free Education in Pathology

Twitter is an excellent source of high-quality pathology material contributed by all cadres of pathology lovers, ranging from medical students on pathology electives to residents, fellows and attendings. Pathologists from all career stages (including post-retirement) and practice settings share their insights and experiences (1).

The topics discussed cover diverse aspects of pathology: gross and autopsy techniques, microscopic findings, specimen photography tips, artifacts, and diagnostic pearls and pitfalls. Discussions also span all pathology subspecialties. These interactions have provided us ample exposure to content areas that are missing in the training and practice of pathology in Nigeria. To put this in context, a 2019 survey assessing pathology services in the country revealed that only a quarter of the 16 participating institutions had immunohistochemistry. Only two had the capacity for other ancillary techniques such as (frozen sections, *in-situ* hybridization and polymerase chain reaction) (12). Therefore, daily Twitter exposure to a variety of cases with robust discussions on ancillary techniques has been of great educational value.

This social media platform particularly shines as a source of up-to-date information, which is essential for all pathologists but more difficult for pathologists working in low-resource settings to access. As a resident and fellow, one of the authors (O.O.F.) and colleagues in other centers can recollect a variety of cases that were hitherto unknown to them until they were encountered on Twitter. Some valuable cases range from seemingly simple ones, such as recognizing the classic "tigroid" background of seminoma on cytology smears (**Figure 2A**) or appreciating the differences between Molluscum contagiosum and Myrmecia warts (**Figure 2B**) to seeing heretofore unheard-of lesions such as the rare colonic mucosubmucosal elongated polyp (**Figure 2C**) (23–30). Some of these cases would later cross our trays not long after we saw them on social media (26, 29). A testament to the immense value of this educational content, O.O.F. stated: "I have learned more pathology from interactions with pathologists and residents on Twitter and Facebook than from physical interactions with people in all my years of training" in an interview, "Education Beyond Borders" by media specialist, Dustin Johnston (31).

Twitter as a Platform for Case Consultation

Most interactions on #PathTwitter are focused on learning through mini case presentations, Twitter-based tutorials (Tweetorials), and quizzes. However, Twitter also provides an avenue to share difficult cases and seek opinions and guidance from those more experienced in certain subspecialties. Almost all Nigerian pathologists are generalists by training and in practice with few having formal subspecialized training. The interest of one of the authors (O.O.F.) in the field of dermatopathology was spawned when a specialist dermatology clinic was set up in his

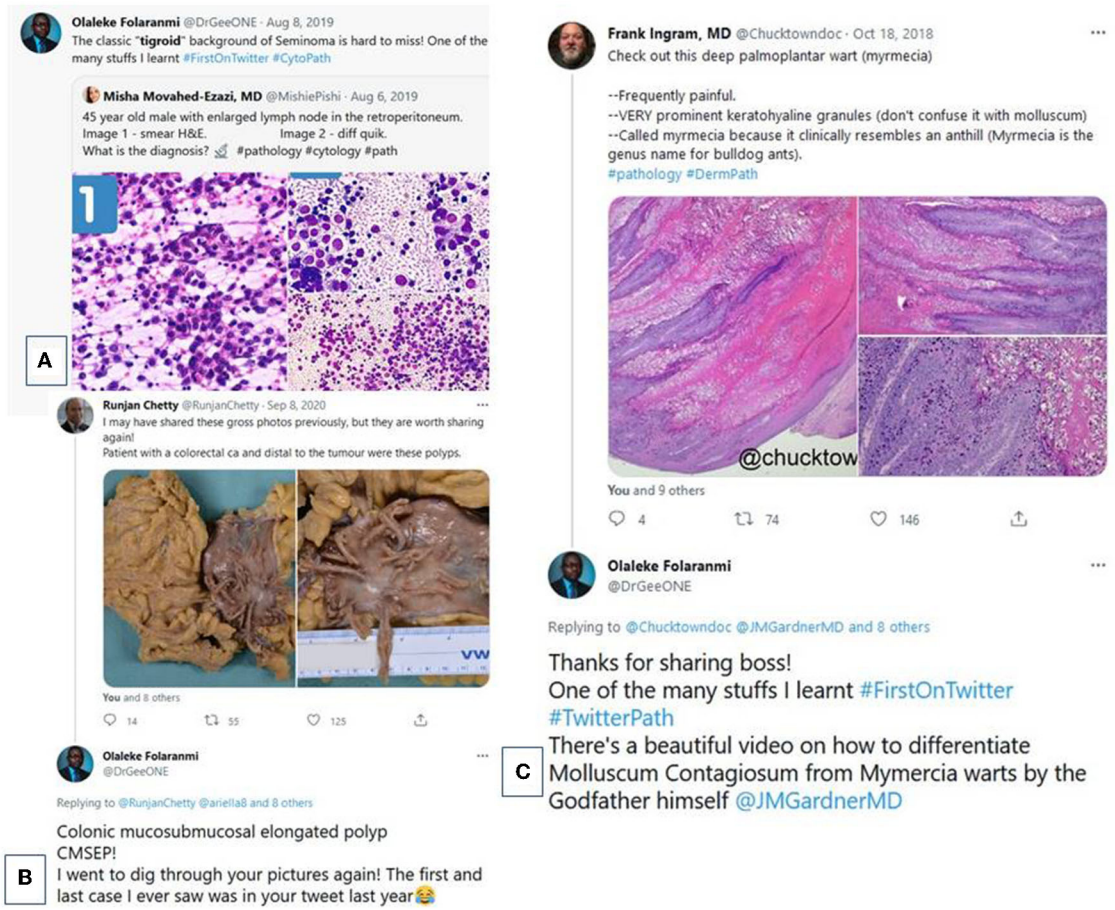

FIGURE 2 | Interactions with educative tweets. **(A)** A quoted tweet from Misha Movahed-Ezazi, MD (@MishiePishi) by one of the authors amplifying one of the many valuable lessons in cytopathology that was learnt first on Twitter: the "tigroid" background of seminoma. **(B)** A tweet by Frank Ingram, MD (@Chucktowndoc) highlighting the salient facts in distinguishing between Myrmecia warts and Molluscum based on morphology. **(C)** A tweet by Runjan Chetty, FRCPC, FRCPath (@RunjanChetty) showcasing an extremely rare lesion 'colonic mucosubmucosal elongated polyp.'

center. There was no known dermatopathologist nearby for the treating physicians to consult, and very few pathologists had an interest in this field as it was perceived to be difficult. Facebook groups (especially the McKee Derm group) and Twitter were the only readily accessible platforms to learn dermatopathology and to share images for guidance. Such unofficial consultations come with an unwritten caveat: the final diagnosis lies with the pathologist who has the glass slides and the clinical history (32). Nevertheless, having access to experts in a particular field who volunteer helpful comments and share opinions on how to approach difficult cases has brought more confidence to our practice (33). The few Nigerian pathologists who are active on Twitter continue to leverage this great opportunity to learn more, bringing the best care available from across the world to patients in Nigeria (33–39).

Technological advancements have greatly improved the way pathologists share cases; glass slides can now be scanned and rendered as high-definition digital images [whole-slide imaging (WSI)]. WSI enables access to the "full picture" of the tissue on the slide, allowing for a more comprehensive case review (40). The adoption of these images is growing on Twitter. Leading here is the "Knowledge in Knowledge Out" [KiKo, (@kiko4docs)] platform founded by Jonhan Ho, MD (@forthejon) to enable physicians share digital images for consultation and teaching purposes.

Twitter as a Niche for Professional Networking and Mentorship

In the traditional sense, most professional networking arises from interactions at physical meetings such as international conferences. Travel grants for people practicing in resource-constrained environments are limited and have become highly competitive. However, in the last few years of consistent interaction with pathologist colleagues on Twitter, we have seen increased followers and a growing interest from around the world in the cases we present. Additionally, these regular interactions have grown into professional relationships leading to invitations to participate in academic activities such as virtual seminars. In 2020, one of the authors (O.O.F.) was selected to be on the team of international screening judges that curate and vote for tweets nominated for PathTweetAward. This Twitter-based,

crowd-funded initiative founded by Sanjay Mukhopadhyay, MD (@smlungpathguy) and Amy Deeken, MD (@AmyHDeekenMD) in April 2018 seeks to promote educational Twitter posts by awarding prizes in open and trainee categories (18). Furthermore, we have seen the effects of Twitter promoting development of "real life" mentors. These mentorship pairings have resulted in increased pathology clinical experience, research publications, and post-graduate training opportunities for Nigerian-trained medical graduates.

The benefits from these interactions and networking have been tremendous: colleagues providing mentorship and inspiring excellence in one's practice, donations of personal equipment, books, and more. Engagement on Twitter has drawn more attention to the knowledge and infrastructure gaps that exist between high-income countries and low- or middle-income areas such as Nigeria (31).

Twitter as a Tool for Research Collaboration

The first published international collaboration generated from and facilitated by a social media post originated in a tweet from Lara Pijuan, MD (@lara_pijuan), a cytopathologist from Spain, who described iatrogenic displacement of cartilage fragments into mediastinal nodes from endobronchial ultrasound-guided fine needle aspiration procedures (41). When the finding was later recognized by another pathologist, it spurred an international collaboration resulting in multiple presentations and publications, including an original research paper (15) as well as a separate manuscript outlining the process of generating and completing a pathology research study through social media (42). The first collaborative project involving Nigerian pathologists through Twitter occurred in 2020. Andrew Schaumberg, PhD (@schaumberg_a), a post-doctoral researcher working on medical machine learning and computational pathology, enlisted the help of pathologists on Twitter for a machine learning project. The project used histologic images from 25 pathologists residing in 13 countries and included two Nigerian pathologists (43, 44). These projects highlight the far-reaching and growing potential for Twitter in promoting global pathology collaborations.

CHALLENGES WITH TWITTER ADOPTION IN NIGERIA

Many pathologists in Nigeria have yet to discover the educational benefits of social media platforms such as Twitter. There are a few reasons why this may be so:

Lack of Awareness

In Nigeria, WhatsApp and Facebook are the top two platforms that are widely used, whereas Twitter ranks 6th (45). This suggests that many physicians may actually have little information on the use of this platform for beneficial academic activities. Our interactions with colleagues further reflect that many Nigerian pathologists do not know that Twitter serves as a hub for medical education and scientific information.

Poor Communication and Infrastructure

Access to internet is still a luxury in Nigeria due to many reasons including: supporting infrastructure deficit, unstable electricity, and the high cost of maintaining equipment, among many others. The number of people with access to the internet is 141 million subscribers, but broadband services have penetrated <50% of these subscribers. These factors have translated to poor internet services and high cost of access (46–48). Furthermore, in order to obtain and share images on social media, investment in a camera and related equipment is necessary and may come at a personal financial cost to the pathologist (31).

Political Unrest

In recent years, more African countries have shut down internet access with most targeting social media sites during political unrest and elections. Since 2015, about 66 countries have restricted access to social media at one point in time, and African countries account for almost half of these (49). In 2021 alone, five African countries had restricted access to internet and social media due to political turbulence. Nigeria announced the suspension of Twitter operations on June 4, 2021 after a week of tense political atmosphere (49–51). This ban was in effect for 222 days, ending at midnight on January 13th, 2022 (52). These restrictions further impair access to information and educational content in a region already struggling with scarce resources.

SOCIAL MEDIA FOR PATHOLOGISTS IN HIGH-INCOME COUNTRIES

The use of social media for professional purposes aligns with the goals of many in high-income countries as well, as it has been documented to positively correlate with traditional measures of gauging academic success and productivity. These include garnering increased recognition and citation of published research works and fostering research collaborations. Indeed, social media impact metrics are being incorporated into promotion and tenure criteria (14).

Beyond these traditional measures of academic success, it is recognized that pathologists, like other physicians, frequently have a desire to teach and engage in outreach. Particularly as the global burden of cancer is rising in prominence and global health initiatives reflect this focus on improving cancer care, (53) the role of pathologists is becoming increasingly more relevant. Pathologists are engaged in global health in a range of manners, including but not limited to engaging in more traditional "on the ground" capacity-building projects to virtual education sessions like the Africa Calls teleconferences that have emphasized camaraderie and educational exchange for 20 years, and virtual global tumor boards (54). Indeed, one of the authors (D.A.K.) has an established interest in helping to develop pathology services in low- and middle-income countries, having engaged in several of the above initiatives and projects in Africa (55). The potential to tap into these opportunities on a small scale in spare moments over an extended period of time has emerged for this author as one of the more compelling reasons to engage in Twitter professionally. Similarly, reflecting on interactions with pathology-interested medical students and

pathology trainees reveals an interest in global health to be relatively common, (56) supporting the idea that there is a pathway for interested parties to promote and develop mutually beneficial global health initiatives, including those that leverage social media. The potential for social media and traditional in-person capacity-building endeavors to synergistically build on one another's progress should be thoughtfully considered as initiatives are envisioned and deployed.

DISCUSSION

Nigeria, and Africa as a whole, faces a number of distinct challenges and gaps in the delivery of quality pathology services. Many of the problems in this region stem from poor economic investment in the healthcare sector, an issue that requires wider economic, infrastructure, and political solutions. However, thoughtful professional social media use has the potential to help ameliorate some of these deficiencies, including improving education and diagnostic pathology services, as well as creating mentorship opportunities and research collaborations.

Pathologists, especially those practicing in low-income countries or in remote communities benefit from insights provided by experts when they share challenging cases on these platforms. Although it is difficult to quantify the impact of these interactions, many have stated that, through the avenues discussed above, professional social media use has positive educational impacts and perceived as improving patient care. A survey in a medical school in Nigeria showed that almost 70% of the respondents are favorably disposed to pathology education *via* social media (21). In the last few years, a few international, predominantly survey-based studies have attempted to measure the impact of the use of social media on dissemination of knowledge in pathology. Results have been overwhelmingly positive in the perception of improved impact as measured through questionnaires and personal experiences; users affirm that teaching cases have improved their knowledge and practice of pathology. Teaching posts, like Tweetorials with embedded pre- and post-tutorial quizzes, have documented increasing learning based on higher percentages of correct answers after the educational post (1, 7, 14, 21, 57, 58). Social media analytics—tools embedded within Twitter—track the impact of a post by documenting impressions (potential viewers) and engagements (viewers that interact with the post). Good engagement rates have been defined (between 1–5%) (58). These metrics reflect the potential reach of the message/tweet and the extent of interaction it generates. They can be useful to provide feedback to content-creators regarding the quality and visibility of their posts. These same metrics inform how journals track the impact of their articles and academics illustrate the reach of a teacher/scholar's work (5, 14, 18). While we are not aware of a specific study to do so to date, social media analytics could feasibly represent a useful tool to help quantify pathology knowledge acquisition in Nigeria.

Potential challenges and limitations also exist for using social media in pathology. One of the most important concerns is protecting the privacy of patients. This is ensured by never including any protected health information such as patient name, age, sex, or date, and location of service. Clinical information should be minimized or altered (1, 5, 7, 18). Sharing of completely de-identified pathology images or other information for educational purposes on social media is legally and ethically acceptable even without patient permission. Legal concerns are understandable; however, no lawsuits based on misuse of social media by pathologists have been filed thus far (6, 7, 18).

Many cases posted on social media are intended mostly for academic purposes. However, pathologists working where there is limited access to ancillary testing or expert consultation services may share cases with diagnostic challenges for assistance. Selection bias and the possibility of only seeing a fraction of the material contained on the actual slide are potential disadvantages to providing opinions on cases shared as a few images on social media. Whole-slide imaging may soon obviate this potential problem but is not yet widely available. Ensuring the accuracy and reliability of medical opinions expressed on social media is also a challenge. However, these platforms open cases up for comments by hundreds of potential "peer reviewers" including experts in the particular field. The resultant constant fact-checking thereby effectively counters false information. Pathologists that share cases for discussion must realize that as the patient's physician, they hold sole responsibility for the final diagnosis and its impact on the patient's care regardless of what other colleagues and experts say. Hence, opinions *via* social media must be regarded as unofficial consultation (1, 7, 18).

Despite the potential benefits, wide reach and availability of these platforms, social media as a tool for advancement of pathology knowledge is still undersubscribed in Nigeria. Contributing factors include poor awareness, unsatisfactory and expensive internet services, and restriction of access to these services during periods of political unrest. Improving awareness of and support for these tools will ideally help mitigate some of the challenges faced practicing pathology in low and middle-income regions of the world.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

OOF, KMI, and DAK contributed to the conception of the work. OOF and KMI wrote the first draft of the manuscript. OAO and DAK provided materials for the initial draft and wrote sections of the manuscript. All the authors contributed to the revision of the manuscript and approved the version submitted.

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SUPPLEMENTARY MATERIAL

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Promoting Best Practice in Cancer Care in Sub Saharan Africa

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Promoting best practice in the management of a cancer patient is rooted in the application of new knowledge derived through various sources including population science, laboratory advances, and translational research. Ultimately, the impact of these advances depends on their application at the patient's bedside. A close collaboration between the oncologist and the pathologist is critical in underwriting progress in the management of the cancer patient. Recent advancements have shown that more granular characteristics of the tumor and the microenvironment are defining determinants when it comes to disease course and overall outcome. Whereas, histologic features and basic immunohistochemical characterization were previously adequate to define the tumor and establish treatment recommendation, the growing capability of the pathologist to provide molecular characterization of the tumor and its microenvironment, as well as, the availability of novel therapeutic agents have revolutionized cancer treatment paradigms and improved patient-outcomes and survival. While such capacity and capability appear readily available in most developed high-income countries (HIC), it will take a concerted and collaborative effort of all stakeholders to pave the way in the same stride in the low and middle-income countries (LMIC), which bear a disproportionate burden of human illness and cancers. Patients in the LMIC present with disease at advanced stage and often display characteristics unlike those encountered in the developed world. To keep stride and avoid the disenfranchisement of patients in the LMIC will require greater participation of LMIC patients on the global clinical trial platform, and a more equitable and affordable sharing of diagnostic and therapeutic capabilities between the developed and developing world. Key to the success of this progress and improvement of patient outcomes in the developing world is the close collaboration between the oncologist and the pathologist in this new era of precision and personalized medicine.

Keywords: low- and lower-middle-income countries, tumor microenvironment (TME), research development-innovation, diversity and inclusion, collaboration pathology and oncology

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THE CRITICAL INTERPLAY BETWEEN PATHOLOGY AND ONCOLOGY

There is no better example of the critical interconnectedness between the pathologist and the clinician than in the field of oncology. Decades ago, the primary support function of the pathologist working with the oncologist was in the diagnostic identification and characterization of an identified malignancy. Categories were rather broad, e.g., carcinoma; sarcoma; myeloma; leukemia; and lymphoma (1) with additional features allowing carcinomas to be categorized into adenocarcinoma vs. squamous cell carcinoma (2). The organ of origin was often deduced based on the histologic features of the tumor on H.E. Stain and supporting clinical as well as radiologic features (3). Advances in immunohistochemistry and the identification of key surface markers have enabled a more nuanced characterization of tumors enabling site of origin as well as well as cellular markers of proliferation that add prognostic significance (4). Advances in molecular testing has added a new layer of capability to identify unique molecular characteristics of the tumor that in turn can provide clues into the molecular mechanism of the malignancy as well as point toward potentially effective therapies based on such predictive markers (5).

More recently, with the advent of NextGen Sequencing (NGS) pathologists have the capability of providing the oncologist critical actionable information directly relevant to the treatment and prognosis of the patient with cancer (6–8). Such advances have shored up the critical interconnectedness between the pathologist and the oncologist. This interplay is best observed at the tumor boards where peer discussion and treatment recommendation is heavily dependent on the contribution of the pathologist, wherein therapeutic decision making is based not just on the histologic features and immunohistochemical markers but in addition on predictive molecular characteristics of the tumor (9).

IMPORTANCE OF THE TUMOR MICROENVIRONMENT (TME)

Stromal processes play a critical role in cancer biology and in some cases, may predict the clinical course of a malignancy better than cellular characteristics of the neoplastic cells itself (10, 11). The tumor microenvironment is a dynamic network that includes the tumor cells, stromal tissue within which are the immune cells, fibroblasts, pericytes, cytokines, and tumor vasculature and the extracellular matrix (12). In addition to epithelial and stromal compartments, the tumor microenvironment contains several cell types of the innate and adaptive immune systems including B and T lymphocytes, dendritic cells, and macrophages (13). This tumor microenvironment (TME) provides added dimension that influences both the biology of the tumor as well as its response to therapeutic efforts and the development of resistance (14). It is integrally involved in nearly all processes critical to the malignant process -tumor initiation, growth, migration, metastasis, and therapeutic resistance (15, 16). As an example, Denkert and colleagues (17) were the first to demonstrate that a high Tumor Infiltrating Lymphocyte (TIL) count in breast cancer tissues was associated with a significant pathologic complete remission (pCR) rate following Neoadjuvant therapy compared to TIL low tumors. Higher levels of TILs also predict for increased responsiveness to chemotherapy independent of estrogen receptor (ER), progesterone receptor (PR), and Human Epidermal Growth Factor Receptor (HER2), with each 1% increment associated with a further increase in the rate of pCR (18).

The role of the pathologist has thus evolved beyond the characterization of the tumor itself and moved on to include the non-clonal cellular microenvironment surrounding the tumor.

IMMUNOTHERAPY AND THE ROLE OF THE PATHOLOGIST

Immunotherapy has become an indispensable arm of cancer management with novel T-cell targeting agents approved by the FDA across various indications both as single agents or in combination with conventional cytotoxic agents (19).

The Nobel Prize in Medicine or Physiology awarded in 2018 acknowledged the important contributions of **James Allison and Tasuku Honjo**, for their pioneering work on cancer immunotherapy (20). Hailed as a revolution in the treatment of cancer, immunotherapy works by boosting the body's natural defenses against cancer. The Nobel committee heralded the efforts of the two scientists as establishing an entirely new principle for cancer therapy by stimulating the inherent ability of our immune system to attack tumor cells (21, 22).

These advances could not have been possible without the investigative role of the pathologist giving importance to not just the tumor cells but to the surrounding TME and tumor infiltrating T-cells (23). The role of the pathologist in investigating the TME in an objective reproducible way is best exemplified by the Programmed Cell Death Ligand combined positivity score (PDL1-CPS)which has become critical as a companion diagnostic tool for the use of a number of novel immunotherapy agents approved by the FDA (24). In the years to come the need for tumor NGS and TME in forging new therapeutic advances will become increasingly evident and critical (25).

CLOSING THE GAP- ACCESS TO MOLECULAR DIAGNOSTICS

The recent advances in molecular diagnostics have contributed to an increasing disparity between patients in the West and those in the developing world where access to such technology remains wanting, both because of lack of trained pathologists with molecular sub-specialization but also as a result of lack of testing facilities within sub Saharan Africa (26, 27). One solution toward bridging this gap is the establishment of collaboration between pathology departments in the LMIC with molecular diagnostic laboratories in the West and the creation of virtual molecular tumor boards both as a platform for peer discussion and capacity

building (28). Part of the impetus for such collaboration may well be the heightened interest in the molecular characterization of cancers diagnosed in the African continent (29). As part of the research collaboration, institutions in SSA could receive access to subsidized Next Generation Sequencing (NGS) of their tumor samples, participation on virtual molecular tumor boards where such cases can be discussed, as well as, opportunity for the patients to participate on clinical trials (30). An important component that would effectively bridge this divide and result in actionable benefit to the African patient would be the availability of novel therapeutic agents for the treatment of patients identified to harbor unique actionable molecular features. Therein lies the opportunity to enroll such patients onto clinical trials that offer access to novel treatments that would otherwise not be available to such patients.

MULTIDISCIPLINARY TUMOR BOARDS AND SPECIALTY CLINICS

Tumor boards represent an ideal platform for data driven peer discussion, both as a means to establish best practice for common cancers, but also as a venue for the multi-disciplinary discussion of complex cases in the context of existing resource limitations (9). The incorporation of peer partners in virtual MDTs from Western academic centers provides an important avenue for sharing new knowledge as well as identifying areas of need for diagnostic investment in the LMIC (31, 32). In addition, such platform provide the opportunity for peer discussion and knowledge transfer that ultimately making the optimal therapeutic choice for the patient.

Beyond the multidisciplinary tumor boards, since January 2021, we have moved to establish specialty clinics dedicated for the multi-disciplinary in-person consultation of patients with specific cancers at the Aga Khan University Hospital Nairobi (AKUHN). We piloted our project with patients presenting with newly diagnosed breast cancer (33). The success of this program resulted in the establishment of a similar platform for patients with newly diagnosed prostate cancer.

The multi-disciplinary in-person clinics go one step beyond the tumor boards and allow the patient to be seen by a team of specialists, including their own primary oncologist. In the case of the multi-disciplinary breast cancer clinic (MBC) and multi-disciplinary prostate cancer clinic (MPC), the consultant team includes a medical oncologist, breast surgeon or urologist, radiation oncologist, and a team of nurses and coordinators. The patient gets to be interviewed and examined by an independent physician member of the team, who then presents the case at the "huddle" where the tumor board discussion and recommendation is revisited in the context of the patient's own input. The management decision from the "huddle" is then conveyed to the patient and family who then have the opportunity to ask questions relating to the entire management plan.

Our experience at the Aga Khan University Hospital Nairobi has demonstrated an enthusiastic embrace of the MBC and MPC model. Interestingly, the multidisciplinary engagement with the patient provides a novel platform to discuss all aspects of the patient management journey in one setting. Our experience at AKUHN has demonstrated that >90% of patients and family members wish to participate in a multi-disciplinary consultation that is provided at no extra charge. Patients get to voice their input and buy-in for the proposed treatment plan. Interestingly, in 20% of the cases, patient preferences result in modification of the treatment plan both in terms of surgical management or selection of neoadjuvant vs. adjuvant therapy. In 10% of cases, patients have desired additional psycho-social counseling and in 10% of cases patients requested nutritional and dietary counseling. A summary of the data collected from the MBC clinics at AKU is provided (**Figure 1**). This platform also serves as an ideal setting to inform patients about clinical trials and to initiate the informed consent process for ongoing research studies including clinical trials.

PROMOTING CANCER CLINICAL TRIALS IN SSA

Africa makes up 1/6 of the world's population (34). Of the 2.7 million clinical trials conducted internationally, less than a fraction of 1% are conducted in the African continent with participation of African subjects (35). This disparity was further unmasked during the Covid-19 pandemic when <2% of the clinical trials linked to the pandemic were conducted in the African continent (36) and African patients had limited access to novel treatment approaches such as Tocilizumab, Remdesivir or participation in convalescent plasma studies. Within Africa itself, nearly 2/3 of the clinical trials are conducted either in Egypt or South Africa with marginal presence in the remaining 44 countries within the continent (35). In the year 2019, of a total of 109 oncology clinical trials initiated in Africa, most of these were conducted in North or South Africa and out of which there were only 6 conducted in Sub Saharan Africa sites (37).

The African continent has long been the platform for studies investigating the prevention and treatment of communicable diseases such as malaria, tuberculosis, HIV etc. However, with the rise in the life expectancy across the continent and lifestyle changes, we have witnessed a rise in the incidence and mortality associated with non-communicable diseases, specifically cancer (38). Establishing clinical trial centers in SSA with well-trained and experienced staff can provide an ideal platform to conduct cutting edge clinical trials in the African continent (39, 40). Conducting therapeutic oncology trial not only levels the playing field for patients in the African continent, provides patients in SSA access to novel therapeutics and allows staff to be trained and experience the unique toxicity profile of such molecules. For the pharmaceutical industry this provides the opportunity to study a patient population with a vastly different ethnic and genetic background, which may well-influence both the toxicity as well as the outcome of such therapeutic agents. Genetic diversity and its impact on drug metabolism has long been demonstrated and may well-impact how African patients tolerate and respond to such novel anti-cancer therapies (41).

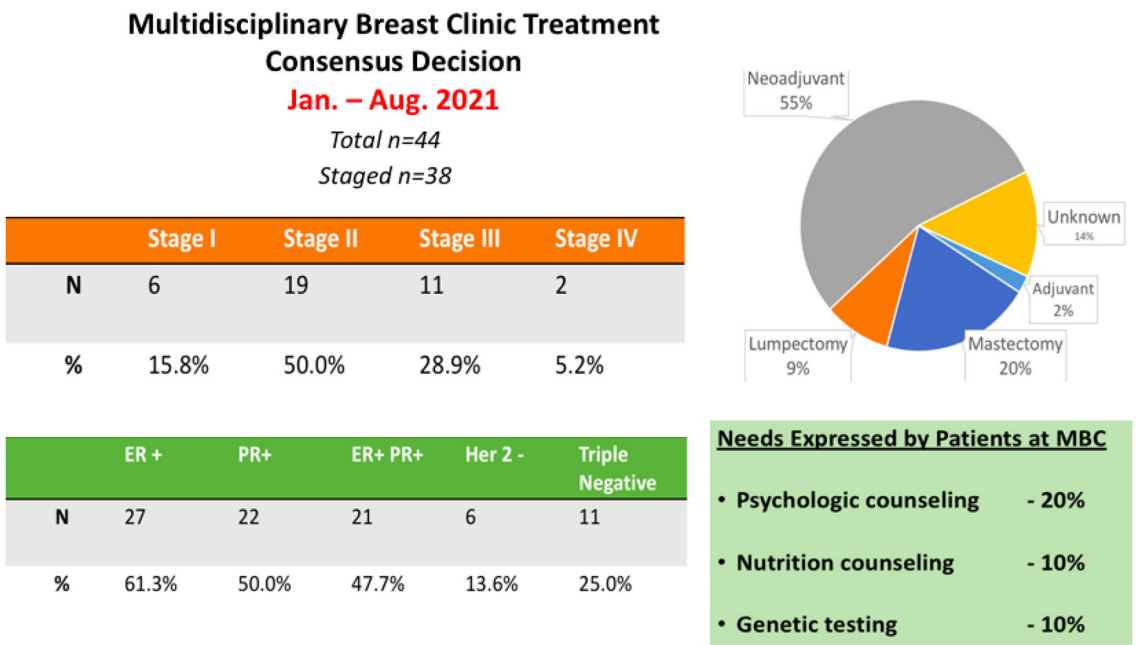

FIGURE 1 | Summary of the data collected from the MBC clinics at AKUHN over a period of 6 months.

"There is increasing recognition of the need for pathologists to be involved early in trial planning and design to ensure methodological rigor in trials requiring sample collection, procurement, storage, transportation and analysis" (42). The recommendations for Interventional Trials 2013 Statement has recently been expanded to include Pathology (SPIRIT-Path) (43). "The guidelines allow investigators to comprehensively address the cellular and molecular pathology aspects of trial protocols, ensuring adequate skills and resources are available at trial commencement and fully leverage the value of biospecimens for translational research" (43).

UNDERSTANDING THE ROLE AND CONTRIBUTION OF GENETIC DIVERSITY

Genetic variations may also be critical in determining effective strategies for the diagnosis and treatment of cancers in patients of African origin given increasing evidence of the molecular diversity of tumors found in African patients as well as a different risk profile as compared to their N. American counterparts (29, 44). As an example, a study of triple negative breast cancer comparing mRNA signatures of women diagnosed with triple negative breast cancer in African women from Kenya revealed a differential expression of both up-regulated as well as down-regulated genes as compared to African American (AA) and Caucasians women from Alabama (45). Similarly, another study comparing AA and European Americans (EA) revealed clear differences in tumor biology between the two groups (46). A recent study looking at AA and EA found significant genetic difference in patients with prostate cancer which may potentially contribute to the incidence and outcome of prostate cancer in these populations (47). It is thus critical that the development of novel therapeutics especially in oncology include the participation of African patients in drug development trials. A current low enrollment of 4% of minority patients in clinical trials conducted in N. America could well be supported and augmented if clinical trial sites in Africa were included (48).

A number of actions would have to be taken if Africa is to become a major partner in drug development. Recent data from the World Bank suggests that African countries invest <1% of their GDP in research and development as compared to USA that spends close to 3% (49). The absence of universal health coverage, the lack of awareness and near absence of cancer screening initiatives and inability to afford standard diagnostic procedures contributes significantly to late presentation and early death among African patients with cancer (38).

BRIDGING THE GAP

Of the 54 countries in Africa only 25 countries have functional population based cancer registries through the AFCRN (African Cancer Registry Network) program that was set up in the year 2012 (50). For decades, the burden, pattern and outcome of cancer in Africa has been understudied. Even functional registries face countless challenges that contribute to the data being inaccurate and unrepresentative. These include generally poor health care infrastructure, lack of a regular and accurate census program, absence of vital statistics, lack of adequately trained personnel and lack of cooperation/contribution from other data sources (51). Computer-based medical information systems also remain underdeveloped (51). Researchers with experience

conducting studies in Africa have opined on the following barriers: lack of infrastructure, financial and human capacity, delays in regulatory and ethical reviews, complex logistical and financial systems (52).

Another major hurdle facing SSA is the lack of training of health care workers in the field of research. Out of 909 training institutions in Africa, only 20% offer training in clinical investigation and research. All of these trainings are at post graduate level and research is not part of the curriculum at the undergraduate level. The vast majority (over 50%) of the training institutions are in Kenya, Ethiopia, SA and Ghana (53).

Despite the large human population and disproportionate burden of disease, Africa lacks the human resources necessary to implement an effective cancer control program. Only 7 African countries have a pathologist to population ratio of more than 1/1,000,000. This is in contrast to 1 pathologist per 15–20,000 population in Europe and N. America (54). A shortage of oncologists also exists in over 25 countries in Africa with clinical oncologist to new patient ratios exceeding >1,000 compared to <150 in the west. It is also astounding to note that ∼8 countries in Africa including Sierra Leone, Burundi and Togo have no clinical oncologists (55). At this stage, unless the paradigm changes, it would take Africa over 400 years to catch up (54). In addition, this disparity is further compounded by the lack of sophisticated molecular diagnostic and treatment capabilities even in those countries that have well-trained pathologist/Oncologists.

In the last decade there have been a number of initiatives by pharmaceutical companies and western nations to establish projects to improve the research capacity, infrastructure and collaboration within SSA (56). Establishment of clinical trials cooperative groups such as those in N. America and Europe will be key to allowing Africa to leap frog and catch up with Western partners. It remains crucial for governments, NGO and clinicians themselves to invest efforts and funds toward the establishment of a more robust cancer prevention and control program in SSA.

As oncology moves from a one size fits all to a more nuanced and personalized approach employing precision medicine technology, the role of the pathologist and interconnectedness with the oncologist, and the close interplay between the pathology bench and the clinical bedside will become increasing important. The pathologist is no longer just a distant consultant involved with the tissue obtained at surgery. Nor is the modern oncologist one who limits him or herself to interpreting the pathology report and prescribing a treatment regime. There is a growing need for the pathologist to become involved at the bedside and understand the nature of the malignant illness, and for the oncologist to better understand and interpret clinical relevance of the technologies applied at the pathology bench. The treatment of the cancer patient of the future will depend on the clinical findings at bedside, interpretation of sophisticated diagnostic studies, and the translation of modern molecular diagnostics results. The successful treatment of the cancer patient will depend heavily on the interconnectedness between the pathologist and oncologist and the availability of novel therapeutic, aided by the availability of clinical trials.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MS was involved with the conceptualization. KS and MS was responsible for writing and editing the original draft. SS was involved with reviewing and editing the final draft. All authors contributed to the article and approved the submitted version.

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BRIEF RESEARCH REPORT
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Building Perinatal Pathology Research Capacity in Sub-Saharan Africa

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Introduction: Over two million stillbirths and neonatal deaths occur in sub-Saharan Africa (sSA) annually. Despite multilateral efforts, reducing perinatal mortality has been slow. Although targeted pathologic investigation can often determine the cause of perinatal death, in resource-limited settings, stillbirths, early neonatal deaths, and placentas are rarely examined pathologically. However, the placenta is a key source of diagnostic information and is the main determinant of fetal growth and development *in utero*, influencing child health outcomes.

Methods: In 2016, our collaborative intercontinental group began investigating infectious perinatal death and adverse child health outcomes in Uganda. We developed and initiated a 4-day combined didactic/practical curriculum to train health workers in placental collection, gross placental examination, and tissue sampling for histology. We also trained a local technician to perform immunohistochemistry staining.

Results: Overall, we trained 12 health workers who performed gross placental assessment for > 1,000 placentas, obtaining > 5,000 formalin-fixed tissue samples for research diagnostic use. Median placental weights ranged from 425 to 456 g, and 33.3% of placentas were < 10th percentile in weight, corrected for gestational age. Acute chorioamnionitis (32.3%) and maternal vascular malperfusion (25.4%) were common diagnoses.

Discussion: Through a targeted training program, we built capacity at a university-affiliated hospital in sSA to independently perform placental collection, gross pathologic examination, and placental tissue processing for histology and special stains. Our training model can be applied to other collaborative research endeavors in diverse resource-limited settings to improve research and clinical capacity and competency for diagnostics and management of stillbirth, neonatal death, and child health outcomes.

Keywords: Uganda, placenta, fetus, histopathology, histology, outcomes, pregnancy, stillbirth

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INTRODUCTION

There are at least 5.5 million stillbirths and neonatal deaths annually (1), and > 40% occur in sub-Saharan Africa (sSA). Despite multilateral efforts, reducing global perinatal morbidity and mortality has been slow, especially in sSA (2). The placenta is the main determinant of fetal growth and development *in utero* (3) and mediates maternal adaptations and maladaptions to pregnancy (4–6), reflecting or influencing a multitude of fetal and child health outcomes (7). One poor outcome is fetal growth restriction. Fetal growth restriction is diagnosed when the fetus fails to meet its full growth potential due to pathological factors *in utero*. Growth restriction most often results from placental dysfunction (8). Globally, fetal growth restriction is a leading cause of stillbirth, neonatal mortality, and childhood and long-term morbidity (8). For these reasons, the International Federation of Gynecology and Obstetrics (FIGO) strongly recommends sending placentas from pregnancies with fetal growth restriction for histopathological examination, and reporting results according to the Amsterdam consensus diagnostic nosology (9). The FIGO recommendation recognizes that placental examination improves diagnostic precision and that the information it provides is often useful when counseling women about the cause of adverse pregnancy outcomes or other obstetric events and for providing guidance on future pregnancies, particularly on the risk of known recurrent placental disorders (8). Furthermore, pathological data on causes of stillbirth, fetal growth restriction, and other undesirable outcomes are necessary to develop appropriate public health initiatives at all levels, plan for clinical services including primary and referral needs, that ultimately will help achieve maternal and child health goals (10, 11).

Unfortunately, though targeted investigation can determine the cause of perinatal death in most cases, stillbirths and early neonatal deaths are rarely investigated using fetal or neonatal autopsy or placental pathology in resource-limited settings (12). Lack of pathology capacity is the main reason placental and fetal examination is rarely performed in sSA. Lack of capacity is multifactorial, including a scarcity of institutions capable of providing training in pathology techniques and research methods and an alarming scarcity of trained pathologists (10, 11). A 2012 survey of pathology resources in sSA demonstrated that all countries except South Africa and Botswana had fewer than one pathologist for every 500,000 people, less than 10% of the pathologist availability in the United States of America (11). Since the 1990s, we have been working in resource-limited settings to build capacity for perinatal pathology and carry out research studies to determine causes of maternal, fetal, and child morbidity and mortality (13–16). Through our individual and collective experience, we have witnessed the dire need to further improve the capacity for placental pathology in sSA.

Here, we report on our program's experience building perinatal pathology capacity at Mbarara University of Science and Technology (MUST) and its affiliated Mbarara Regional Referral Hospital in southwestern Uganda. Recognizing that human resources for pathology training and research are limited in this setting, as elsewhere in sSA, we leveraged a 20-year multilateral research and teaching partnership involving MUST, Massachusetts General Hospital (MGH), and, more recently, Seed Global Health, to train a cadre of health workers and laboratory technicians to perform gross and histologic placental examination. We detail the structure of the training program and its outcome including quality of prepared slides and identified pathologic placental diagnoses.

METHODS

In 2016, our collaborative intercontinental research group comprised of investigators and educators affiliated with MUST and MGH began a research program investigating infectious causes of stillbirth, early neonatal death, and adverse child health outcomes in Uganda. One team member (FAM), a perinatal pathologist from the United States with specialized training and extensive perinatal pathology experience, was stationed full-time on site at MUST for over 2 years, a volunteer position facilitated by the Seed Global Health program. Seed is a not-for-profit organization focused on human resource for health capacity-building in sSA through sustained collaborative engagement.[1] Another team member (LMB), an infectious diseases physician and intensivist trained in the United States, lived on-site for 3 years coordinating the program's development and carrying out research projects with a local obstetrician collaborator (JN). A third team member (DJR, an experienced perinatal pathologist) traveled to the site several times per year to provide the on-site team with support from the MGH Pathology Department and assist with building program capacity, especially training of a local team member (AB) in immunohistochemistry techniques.

Before initiating this program, placentas in Mbarara were rarely examined at birth for either pathologic gross or histologic changes. We began our initiative by developing a training program in gross placental examination and sampling for histologic review, starting with a 4-day combined didactic/practical curriculum to train nurses, midwives, and junior physicians in placental collection and gross assessment to identify lesions that would need sampling for histology. An example training schedule is provided in **Table 1**, which consisted of combined didactic presentations on placental function and structure and hands-on training in gross placental examination, umbilical cord blood sampling, placental dissection, and sampling of placental disc, membranes, and umbilical cord, and formalin-fixation of samples and sample trimming for histopathology block creation. Often, this training program was embedded in a larger training curriculum on research ethics and principles of informed consent along with additional procedures and data collection relevant to placental and maternal-child research. We employed a train-the-trainer model, with two experienced perinatal pathologists (DJR, FAM) who trained junior pathologists (including RA) and one junior physician scientist (LMB), who further trained local health workers. After carrying out placental gross examination and sampling procedures for at least 2 months, local health workers were then

[1] https://seedglobalhealth.org/

TABLE 1 | Training program in gross placental examination and sampling for health workers.

Day (total time)	Training activity	Type of activity	Participants and roles	Activity duration
Day 1 (4–6 h)	Introduction to placental anatomy, physiology, and pathology	Didactic (computer slide-based or video lecture)	Trainer-led talk attended by trainees	45 min
	Overview of placental procedures: • Gross placental examination • Sampling umbilical cord blood • Gross placental pathology • Sampling placental disc, membranes, umbilical cord • Formalin fixation and trimming	Didactic (computer slide-based or video lecture)	Trainer-led talk attended by trainees	1 h
	Demonstration of gross placental examination and sampling	Hands-on practical	Trainer-led, attended by trainees	2 h
	Practice gross placental examination and sampling	Hands-on practical	Trainees, observed and coached by trainer	1–2 h
Day 2 (3–5 h)	Demonstration of placental specimen trimming	Hands-on practical	Trainer-led, attended by trainees	45 min
	Practice placental specimen trimming	Hands-on practical	Trainees, observed and coached by trainer	1–2 h
	Practice gross placental examination and sampling	Hands-on practical	Trainees, observed and coached by trainer	1–2 h
Day 3 (2–4 h)	Practice gross placental examination and sampling	Hands-on practical	Trainees, observed and coached by trainer	2–4 h
Day 4 (3–5 h)	Practice gross placental examination and sampling	Hands-on practical	Trainees, observed and coached by trainer	2–4 h
	Practice placental specimen trimming	Hands-on practical	Trainees, observed and coached by trainer	1–2 h

capable of training peers. The training program was modified during the COVID-19 pandemic to include remote live video didactic sessions (conducted by LMB) paired with in-person peer training (conducted by prior trainees with > 2 months of hands-on experience). One author (DJR) also separately facilitated training a histology technician (AB) to perform immunohistochemistry. This focused training program consisted of methods to prepare tissue for immunohistochemistry, battery testing, quality control and assurance practices, antigen retrieval, direct and indirect methods of manual immunohistochemistry staining, and development of a standardized operating protocol for immunohistochemistry.

Research grant funding (to LMB) and a volunteer stipend (to FAM) paid for time spent training health workers in gross placental examination and sampling. Histopathologic and histopathology supplies were funded by research grant funding (to LMB) as well as departmental partnership funds at MGH (to DJR and others). Gross placental examination findings were recorded on a standardized case report form developed by DJR in accordance with her practice and training at Harvard Medical School teaching hospitals. Histopathology findings were recorded on standardized case report forms using the categories defined in the Amsterdam consensus diagnostic nosology (9). Case report forms were then abstracted into a Research Electronic Capture (REDCap) database (17) for analysis.

All research participants provided written informed consent to participate in each research study. The first research study was approved by MUST (12/11-15), Mbale Regional Referral Hospital Research Ethics Committee (082/2016), Partners Healthcare (2016P000806), and Pennsylvania State University College of Medicine (STUDY0004199). The second research study was approved by MUST (11/03-17) and Partners Healthcare (2017P001319). The third research study was approved by MUST (10/06-19) and Partners Healthcare (2019P003248).

RESULTS

Placental Gross Assessment and Sampling

Training was conducted for three distinct studies over a 5-year time span from 2016 to 2021 (**Table 2**). One training program per study period was deemed sufficient to train all staff, with the exception of Study 3, when training was carried out in stages to accommodate newly hired staff. Refresher training was also provided due to interruptions in the research program from the COVID-19 pandemic. Overall, we trained 12 health workers, including six midwives, four nurses, and two junior medical doctors. All trainees are now capable of independently performing gross placental examination and collecting samples for histopathologic examination for research and/or clinical purposes (**Figure 1**). Altogether, the Mbarara trainees have collected, and performed gross placental assessment, on over 1,000 placentas to date (**Table 2**). Approximately 80% of all histologic slides prepared from these

TABLE 2 | Health workers trained, number of placentas processed and evaluated, and gross and histologic pathology findings in three separate research studies that constitute the foundation of perinatal pathology research at a Ugandan regional referral hospital.

Research project **Participants**	**Project 1** ***n* = 100**	**Project 2** ***n* = 352**	**Project 3*** ***n* = 600**
Years carried out	2016–2017	2017–2018	2019 –
Number of newly trained health workers	4	2	6
Health worker qualifications	Junior medical doctor (1) Nurse (2) Midwife (1)	Junior medical doctor (1) Midwife (1)	Nurse (2) Midwife (4)
Number of returning, previously trained health workers	–	2	3
Placentas evaluated, *n* (%)			
Gross examination	100 (100)	352 (100)	525 (87.5)
Histopathology	100 (100)	352 (200)	118 (19.7)
Number of placental parenchymal histopathology slides per participant case, median (IQR)	2 (2–3)	2 (2–3)	4 (4–4)
"Adequate" histopathology quality, *n* (%)	100 (100)	316 (89.8)	87 (73.7)
Immunohistochemistry (IHC) performed, *n* (%)	0	51 (14.5)	0
Placental weight in grams, median (IQR)	Mean 425	456 (382–529)	443 (375–511)
Placentas < 10th percentile of expected weight for gestational age (*n* = 616)		205 (33.3)	
Histopathology findings			
Acute chorioamnionitis (*n* = 561)		184 (32.3)	
Maternal vascular malperfusion (*n* = 453)		115 (25.4)	

**Study still in progress. Not all data for all studies were available to be included.*

placentas were of adequate quality for diagnostic interpretation. Median placental weights ranged from a mean of 425 grams in Study 1 to a median of 456 (IQR 382–529) grams in Study 2 and 443 (IQR 375–511) grams in Study 3 (**Table 2**). Overall, 33.3% of placentas were in the < 10th percentile in weight, corrected for gestational age using a standard weight chart (18).

Placental Histopathology

Trainees fixed and trimmed formalin-fixed samples of umbilical cord, membranes, placental parenchyma, and any focal disc or umbilical cord lesions to create formalin-preserved tissue specimens for paraffin embedding. In total, 5,408 tissue samples were processed and used for research diagnostics through the three studies. Grant funding supported local histology technicians (AB and four others) to create paraffin-embedded blocks, hematoxylin and eosin (H&E)-stain slides and generate slides for routine histopathology. Due to human resource constraints on local pathologists, slides were interpreted by perinatal pathologists based in the United States (DJR, FAM, and CKC) according to the Amsterdam consensus diagnostic nosology (9). Common histologic diagnoses included acute chorioamnionitis (present in 32.3% of cases), and features of maternal vascular malperfusion (MVM, present in 25.4% of cases).

Publications and Other Deliverables

To date, we have published three manuscripts reporting the results of this work (14, 15, 19), with one more currently under review and another three manuscripts in preparation. The local technician (AB) trained to perform immunohistochemistry staining on placental tissue for research (14) and clinical purposes has leveraged these skills to perform immunohistochemistry staining to assist in diagnosis of breast and other cancers.

DISCUSSION

Through a structured and targeted training program, we built capacity at a university-affiliated hospital in sSA to independently perform placental collection, gross pathologic examination and placental tissue processing for histology and special stains for placentas of diagnostic interest, adequate for research purposes. The health workers trained came from a variety of medical specialties including general nursing, midwifery, pediatrics, obstetrics and gynecology, and pathology. The training program demonstrated that health workers with diverse backgrounds can learn and teach essential placental gross examination and sampling techniques for histopathology. During the social disruption of the early COVID-19 pandemic, we hybridized our training model to include a virtual component that can be applied to other collaborative research endeavors in different resource-limited settings. We advocate for the *train-the-trainer* model to ensure ongoing local development of trainee skills to carry out research, improve on-site clinical diagnostic capacity and patient care, and allow trainees subsequently assume trainer roles to train others going forward.

We found a high proportion (33.3%) of placentas that weighed < 10th percentile of expected weight for gestational age. However, gestational age correction was performed using a standard weight chart based on women in the northeastern United States of America (18) since Ugandan or sSA-specific placental weight charts are not currently available. We believe that placental weights in Uganda appear smaller for gestational age when high-income reference standards are used, likely due to the high percentage of small placentas by weight. Furthermore, measurements aggregated from seven studies in high-resource settings reported a median weight of 520 (10–90% range 408–642) g (20). Thus, gestational age correction using non-local standards may lead to overdiagnosis of low

FIGURE 1 | Trained health workers independently dissecting and sampling placenta after gross examination in Mbarara, Uganda.

placental weight. However, low placental weight could also indicate placental hypoplasia, a feature of MVM. Combined with small placental size, a relatively high prevalence MVM (25.4%) may point to a relatively high incidence of other features of MVM, including placental infarcts, increased syncytial knots, distal villous hypoplasia, increased perivillous fibrin, etc. Together, these findings are concerning indicators of possible intrauterine fetal growth restriction, which is a leading global cause of stillbirth, neonatal mortality, and morbidity (8). Furthermore, maternal HIV infection and antiretroviral treatment are associated with MVM, with prevalence as high as 30–40% (21). Large-scale perinatal pathology studies carried out in sSA have also demonstrated associations between utero-placental vascular pathology, acute chorioamnionitis, and stillbirth (22, 23). These results, reflecting a miniscule proportion of all deliveries in sSA, highlight the need for histopathological examination of placentas of unexplained stillbirth deliveries, fetal growth restriction, and maternal HIV infection, and the need to use standardized criteria for reporting diagnoses in order to compile and compare results across various settings (22). Alongside developing local placental weight charts, the high prevalence of low placental weight should be further investigated and addressed to improve mortality in children under age five.

Though largely successful, our initiative has several limitations. Only approximately 80% of the slides produced by our trainees were adequate for histopathologic interpretation. The 20% that were inadequate often had issues with processing and fixation but did not have issues with trimming/cutting of specimen tissue or paraffin blocks, or staining. The large proportion of inadequate slides emphasizes the continuing challenge of consistent slide production in resource-constrained settings. Furthermore, although local pathologists based at MUST and its affiliated regional referral hospital are capable of interpreting H&E-stained placental slides, unfortunately, human resource limitations currently limit their capacity to add placental cases to their current practice and prioritize these cases over other important diagnostic applications (e.g., new tumor diagnosis). In our future work, we aim to help define priority clinical cases for placental analysis and build capacity to perform on-site fetal autopsy. In addition, although we trained one histopathology technician to perform immunohistochemistry on placental specimens and other tissues, there remains limited local capacity to routinely perform special stains due to a lack of supplies and funding. However, the acquired immunohistochemistry skills can be readily useful for future research endeavors and clinical projects when supplies are available. Additionally, valuable skills may be passed on to other trainees thereby augmenting the pool of skilled individuals able to perform such tasks.

In the future, we hope that increasing research capacity will also translate into greater use of perinatal pathology for clinical diagnosis and management of stillbirth, neonatal death, and child health outcomes in sSA. Information gained from autopsies of stillbirths and neonatal deaths is essential to designing interventions to improve outcomes. Minimally invasive approaches should be considered as alternatives to conventional autopsy, which are proven acceptable alternatives to conventional pediatric autopsy in resource-limited settings (24). Toward this end, building perinatal pathology research capacity also contributes skills and resources that can have a positive collateral impact on patient care. Some strategies to address the shortage of pathology services include concentrating specialists in tertiary care centers and establishing structured referral systems to improve access. Digital telepathology has also been proposed as a stopgap measure to address the pathologist shortage in sSA, with some success, largely when embedded within long-term international collaborations (25). Multinational partnerships can also help with human resource limitations by providing training and diagnostic consultation, including virtual training, which has proven successful in some settings (26). A first step would be to survey current capacity and develop a tool for assessing needs. Although we report our experience of limited capacity to carry out perinatal pathology service in sSA, the true capacity is currently unclear. Recent efforts to assess anatomic pathology capacity have largely been focused on cancer diagnosis. Thus, regional surveys of perinatal pathology capacity should also be implemented.

Collaborative international research programs can, and should, contribute to clinical and research pathology capacity development in sSA (27). There are several good examples of successful programs in addition to ours, including a pathology program at Anokye Teaching Hospital that became self-sustaining after an 11-year partnership with University Hospital of North Norway and could serve as a model for others. The Anokye Pathology Department now provides surgical pathology, cytology, immunohistochemistry, frozen section services, and residency training, fully independent

from international assistance (28). A similar partnership between the Fred Hutchinson Cancer Research Center at the University of Washington and Makerere University and its associated Mulago Hospital led to the establishment of a clinical pathology laboratory at the Uganda Cancer Institute that handled 5,700 tissue diagnoses in 2019 and routinely offers immunohistochemistry services (29). Though these examples are in the cancer field, similar programs could, and should, be established for clinical placental pathology and fetal autopsy. In settings where human resources are especially limited, the ability to conduct a thoughtful autopsy and having a keen eye for identifying gross placental lesions are key elements of perinatal pathology, and training health workers to develop these skills should be prioritized. In settings where histopathology is not available, even weights, measurements, and a thorough gross examination can be critically important, and on occasions sufficient to identify the potential cause of fetal or neonatal death (10).

In conclusion, we provide one example of a successful perinatal pathology research program in sSA that could serve as a model for others, increasing perinatal pathology capacity for both clinical and research applications. Furthermore, we will strive to continue our collaborative partnership for many years to come, building further capacity in clinical diagnostics to improve pregnancy and child health outcomes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Mbarara University of Science and Technology Institutional Review Committee, Mbale Regional Referral Hospital Research Ethics Committee, Partners Healthcare, and Pennsylvania State University College of Medicine Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LB: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, writing—original draft, and writing—review and editing. JN: conceptualization, data curation, investigation, methodology, project administration, resources, supervision, validation, and writing—review and editing. FM: conceptualization, data curation, histopathology slide interpretation, formal analysis, investigation, methodology, project administration, supervision, and writing—review and editing. CKC: conceptualization, data curation, histopathology slide interpretation, supervision, and writing—review and editing. AB and RA: data curation, methodology, project administration, supervision, validation, and writing—review and editing. VK: conceptualization, supervision, and writing—review and editing. DR: conceptualization, data curation, histopathology slide interpretation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, and writing—review and editing. All authors agreed to be accountable for the content of the work.

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Pathologists Overseas: A volunteer-based model for building sustainable, high-quality pathology and laboratory medicine services in low- and middle-income countries

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For thirty years Pathologists Overseas (PO) has worked in low- and middle-income countries (LMICs) to provide affordable, sustainable, and high-quality pathology and laboratory medicine (PALM) services through strategic partnerships and the efforts of our large volunteer network. We address low quality diagnostic services by targeting the 3 pillars of PALM quality: human resources, systems, and quality and accreditation. To improve human resource capacity, PO and our partnering organizations provide virtual continuing education to pathologists and laboratory professionals in these countries. To improve systems, we provide laboratory information system installation and implementation support. Lastly, to improve quality and help laboratories progress toward accreditation, we support an external quality assurance program for laboratories in LMICs. As a relatively small organization, PO demonstrates that a network of dedicated volunteers, in partnership with corporations and professional organizations, can initiate sustainable change in the quality of PALM services in LMICs by focusing efforts on the core components of laboratory quality.

KEYWORDS

global health, health systems strengthening, low- and middle-income countries (LMICs), pathology, laboratory medicine

Introduction

Pathology and Laboratory Medicine (PALM) are required for detection, management, and prevention of disease and thus are essential components of the healthcare system. Yet, there is broad consensus that the quantity and quality of these services in many low- and middle-income countries (LMICs) are poor (1–3). There is less consensus on how to improve the state of PALM in these countries. Pathologists Overseas (PO) is a US-based, volunteer-run organization whose mission is to improve PALM where resources are limited. Herein, we describe the work we have undertaken that has been most effective in improving practice in partnering laboratories in LMICs through a framework of PALM quality.

Before we introduce the framework, we want to share that our approach is focused on strengthening health systems rather than addressing any single disease. Historically, many global health programs, including those in PALM, have taken a "vertical" approach, where funding is channeled toward specific diseases (e.g., tuberculosis, malaria), without direct investment to strengthen the foundation of health systems (4). PALM as a discipline, regardless of disease entity, requires 3 things in order to render consistently accurate, reliable, and reproducible test results and pathologic interpretations. These are infrastructure, qualified staff, and a legal and regulatory environment that supports consistent quality standards. Building this capacity takes coordinated investment that has been historically lacking in LMICs (5).

Framework

To this end, Pathologists Overseas (PO) has over thirty years of experience working in LMICs and approaches building laboratory capacity by targeting the 3 pillars of PALM quality: *human resources, systems, and quality and accreditation*. As a small organization, our mission is to provide affordable, sustainable, and high-quality diagnostic services in LMICs through strategic partnerships and the efforts of our large volunteer network. In order to demonstrate how a volunteer-based model can be utilized to build PALM capacity, we provide a brief history of PO followed by an overview of our current efforts in the framework of human resources, systems, and quality and accreditation.

History of pathologists overseas

Dr. Heinz Hoenecke founded PO in 1991 with the mission to affordably and sustainably improve PALM services in LMICs through the efforts of volunteer pathologists, technologists, and laboratory scientists. As PO grew, it became evident that this mission would be better served by incorporating as a non-profit organization. Despite this formality, PO remained entirely volunteer-run for the next 30 years during which time it has organized and funded major projects in Kenya, Eritrea, Nepal, Ghana, Madagascar, Bhutan, Peru and St. Lucia as well as more limited endeavors in over 20 countries (6). Overall, these efforts can be summarized as establishing new technologies, initiating comprehensive quality control programs, upgrading laboratory facilities, providing textbooks and educational materials, developing training programs, and facilitating placement of physicians from LMICs to pathology fellowships in high-income countries (HICs).

PO's prior anatomic pathology (AP) capacity building efforts include our first initiative in Kenya where we provided histopathology services to more than 40 mission hospitals for over 5 years through the support of over 90 volunteers. In keeping with the goal of transitioning services to local leaders, PO transferred all revenue and equipment to A.I.C. Kijabe Hospital outside of Nairobi. To meet the surgical pathology need in Madagascar, PO worked with local stakeholders to establish a histopathology lab and recruit volunteers from HICs to fill in service gaps and train local physicians. Two of these local physicians ultimately took over the clinical service and laboratory operations. In Ghana, PO was invited to establish a histopathology lab and pathology training program at Komfo Anokye Teaching Hospital. Similar AP capacity building projects were undertaken in Nepal and Peru.

Starting in 1994, PO's efforts expanded to building clinical laboratory capacity. In Eritrea, we established a country-wide Laboratory Quality Assurance System, expanded testing to include clinically necessary care (e.g., hemoglobin A1c for diabetes), established a diabetes clinic, and helped launch a medical school. These efforts were supported for over 10 years and the Laboratory Information System (LIS) we installed has been sustained for 20 years. In Bhutan, we installed LISs that are now supporting over 20 hospitals/clinics in each county.

Although each setting was unique, there were pervasive challenges such as unreliable staffing, supply chains, and quality assurance programs, which led us to focus our work in 3 major areas. The first was expanding human resource capacity through the provision of technical support, historically by deploying volunteers to laboratories. The second was building sustainable infrastructure through the installation and continued support for LISs. The third was improving quality and moving laboratories toward accreditation by enrolling and mentoring them in External Quality Assurance (EQA) programs. These historical approaches, especially the deployment of volunteers for short-term opportunities, represented our primary strategies to build capacity for most of our history. With the advent of the COVID-19 pandemic as well as an acknowledgment of the improving landscape of staff capacity in LMICs (**Table 1**), we

TABLE 1 Change in pathologists and pathology trainees from 2015 to 2022.

Region (Number of countries)	Countries with pathologists (n = 40)		Pathologists			Pathology trainees		
	2015	2022	2015	2022	Δ (%)	2015	2022	Δ (%)
Francophone (17)	16	17	100	155	+55 (+55)	37	128	+91 (+250)
West Africa (5)	2	5	163	148	−15 (−10*)	220	120	−100 (−45)
East Africa (9)	8	9	183	290	+107 (+58)	97	171	+74 (+76)
South Africa (9)	8	8	280	336	+56 (+20)	104	148	+44 (+42)
Total	34	39	726	929	+203 (+28)	458	567	+109 (+24)

*Totals for West Africa reflect institution-based pathologists and do not represent the number of pathologists who have moved to private laboratories.

have pivoted toward virtual activities with the goal of reaching a broader audience and increasing the number of volunteers we are able to engage in a cost-effective fashion. These virtual activities include continuing education programs to support this new class of pathology and medical laboratory professionals, and digital content, such as podcasts and blogs, designed to support the flourishing of the LMIC PALM community.

Three pillars of palm quality

Human resources

The first of the three pillars of PALM quality is human resources. A significant barrier to providing and advocating for quality services has been and continues to be a lack of trained PALM professionals in many LMICs. From 2012 to 2014, the African Strategies for Advancing Pathology, a group made up primarily of pathologists from 7 African countries and their partners in the US, Europe and Australia, performed a survey of pathologists and trainees in sub-Saharan Africa (SSA) (7).

The survey demonstrated that, with the exception of South Africa, SSA countries had less than 1 pathologist per million people. In addition, there were no pathologists in 6 countries and only 18 active training programs in the 40 SSA countries surveyed (7). While there is no clear guidance from international health organizations indicating the number of pathologists per capita (anatomic and clinical) required for functional health systems, the number in resource-limited countries is clearly insufficient. Exacerbating the staffing shortage is a concomitant scarcity of trained laboratory technologists, histotechnologists, and cytotechnologists.

Since 2015, there has been a significant effort by national, regional and international organizations and academic institutions to increase the number of pathologists and trainees (**Table 1**) (8). Nigeria, Uganda, Democratic Republic of Congo, and Cuba supply pathologists in other countries with no or inadequate national staff (8). Furthermore, at least 15 countries have post-graduate trainees in other countries, mainly South Africa, Kenya, Tanzania, Uganda, Nigeria and Côte d'Ivoire.

One key component to maintaining a knowledgeable PALM workforce is access to continuing education programs (CE), which are limited in many LMICs (3, 9, 10). To address this gap, previous groups have executed successful in-person CE programs for pathologists, laboratorians, and clinicians (11–13). Although in-person activities allow for enhanced interactions between participants and trainers, they are costly to implement, challenging to scale, and may be less accessible to learners in rural areas or areas with restricted travel.

Adapting CE content to a virtual format represents a solution to these barriers (14). This need to adapt was further accelerated by limitations caused by the COVID-19 pandemic. As a small, volunteer-based organization, providing high-quality, virtual CE represented a novel strategy to synergize PO's mission of increasing human resource capacity through engagement of our volunteer network during a time of limited international travel and collaboration with other partners active in global PALM.

In Spring 2021, we partnered with the American Society of Clinical Pathology (ASCP) to offer a free 10-week Laboratory Quality Management Systems (LQMS) course based on the World Health Organization (WHO) LQMS Essentials. An initial needs assessment included data from over 200 laboratorians representing 63 institutions, predominantly in SSA. The purpose of the needs assessment was to understand the professional background and work setting of potential participants in order to customize the content to their needs. We recruited 15 trainers from our volunteer network to virtually deliver lectures and illustrative cases to participants.

In 48 hours, 617 individuals representing 156 institutions across 24 countries registered for the course. Over 99% of registrants (n = 614) were from countries in SSA. Registrants represented all major areas of the laboratory (e.g., histology, microbiology, transfusion, chemical pathology, etc.), held a variety of positions from laboratory technician to consultant pathologist, and over 70% worked in the public sector. Live attendance at each of the 20 sessions ranged from 150 to 200 participants with many more individuals accessing the lecture recordings and materials available for free online following the course's conclusion.

We received overwhelmingly positive feedback from the 180 participants who filled out the post-course survey. The majority of participants' self-reported comfort and knowledge level increased in all LQMS areas following the course and over 95% were interested in taking another virtual course. Of the topics covered, participants expressed interest in receiving additional content in leadership (50.8%), process control (48.6%), and process improvement (42.9%). Moreover, several participants would have appreciated a more interactive format.

In response to this success, PO recently partnered with BIO Ventures for Global Health (BVGH) and Ahmadu Bello University Teaching Hospital (ABUTH), to launch a free 8-week virtual course on pediatric and lymph node pathology in February 2022. Representatives from PO and ABUTH developed the curriculum, recruited lecturers from our volunteer network, and moderated the virtual sessions. BVGH hosted the lectures and course content, provided administrative and technical support, and coordinated all CE credits with partnering institutions in sub-Saharan Africa. Similar to our prior experience, over 700 individuals representing 19 countries in SSA registered for this series. The average attendance at the live lectures was 219. Participants shared that they felt more confident rendering the correct diagnosis as a result of the course and that they planned to share the information they learned with their colleagues. PO is currently collaborating with ASCP and Heart to Heart International (HHI) to adapt the virtual LQMS course for a Spanish-speaking audience in Central and South America in summer 2022.

The PO experience highlights the need and enthusiasm for accessible, high-quality CE in LMICs. It also illustrates how relatively small organizations can enhance their impact on the communities they serve through utilization of a scalable platforms, partnerships with in-country institutions and like-minded organizations in HICs and leveraging a network of interested volunteers. We plan to continue offering free virtual CE as one strategy for addressing the human resource gap. However, we hope to explore creative ways of incorporating case-based interactive sessions into our virtual curriculum and to supplement our efforts with focused and strategic in-person training workshops.

Systems

The second pillar of laboratory quality is systems. The operation and management of modern clinical laboratories is a sophisticated and complex process, the success of which requires adequate physical and technical infrastructure. One example are LISs, which play a crucial role in streamlining this workflow, reducing errors, and enabling quality indicator monitoring (15). In HICs, laboratories typically initiate the process of LIS implementation by carefully selecting a software that meets the laboratory's unique clinical and non-clinical needs, the latter including regulatory and billing requirements and financial and infrastructure constraints. Once installed, LISs will ideally operate with minimal disruption and access to rapid resolutions, should problems arise. In HICs, this is accomplished through the availability of on-site experts trained in information technology (IT) and LIS support. Given the above considerations, the implementation and maintenance of LISs is a daunting undertaking for most laboratories in LMICs.

PO has recognized this system challenge since its inception. After forging a strategic partnership with a US-based LIS Company, Comp Pro Med, PO has successfully supported LIS implementations in partnering public laboratories in Bhutan (n = 1), Eritrea (n = 1), Ethiopia (n = 1), Malawi (n = 1) and Nigeria (n = 2). Inspired by the initial successes, Bhutan and Ethiopia have since independently expanded the system to 8 and 25 laboratories, respectively. From a sustainability perspective, all LIS implementations except one continue to operate successfully and sustainably. At least one of the laboratories has maintained their LIS for 20 years.

PO continues to engage with laboratories interested in LIS implementation support through our rigorous vetting process. This process involves scrutinizing the capacity and commitment of interested laboratories, at an institutional and departmental level, to implement and independently sustain the system (**Figure 1**). Most recently, PO partnered with Comp Pro Med, HHI, and LIS experts from Ethiopia and Nigeria, to install an LIS at Ahmadu Bello University Teaching Hospital (ABUTH) in Nigeria. As of this writing, there are 5 pending LIS requests.

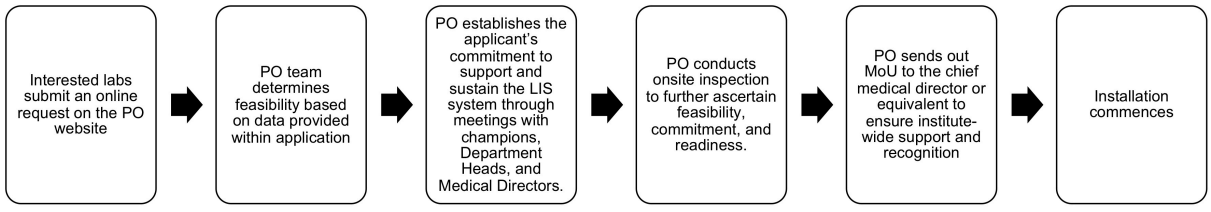

FIGURE 1
Flow chart illustrating process for vetting laboratories interested in LIS implementation.

In summary, the cost of LISs can be prohibitive for many clinical laboratories with limited resources, precluding implementation of these systems despite their importance in laboratory operations. Though limited in scope, PO has adopted an approach that focuses on mentored LIS installation and maintenance in its ongoing efforts to improve clinical laboratory infrastructure in LMICs.

Quality and accreditation

The third pillar of laboratory quality is quality and accreditation. In HICs, an essential and highly regulated component of quality and accreditation is external quality assessment (EQA), which is a system of ensuring the comparability and continued monitoring of clinical test results across different laboratories and over time (16–18). Typically, EQA providers offer performance reports that score participants' results compared to assigned target values and results from peer groups, but there is wide variation in terminology, interpretation, and performance expectations globally (19). Laboratory professionals are often faced by the availability of numerous commercial programs, difficulty in communication with EQA providers, and lack of a sufficient number of participating peer groups for a particular measuring system (20). All of these and the additional obstacles faced by clinical laboratories in low-resource settings must be grappled with in the implementation and oversight of an EQA program in LMICs. This has led to heterogeneity in the scope and success of participation in EQA programs, and even these tend to be restricted to specific analytes or diseases (21–24).

PO has had a long and productive partnership with the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) to enroll participants, free of charge, in the compact general chemistry program. This program consists of 6 surveys mailed at the beginning of the year with set dates for result submission for each survey over the subsequent months. Each survey includes two levels of commonly ordered tests which are scored on comparability to target and peer-group results. Since 2009, PO has enrolled up to 24 laboratories from 6 countries in this program with the majority of labs participating for at least three years and one lab continuing to participate since enrollment in 2014. Between 2009 and 2014, PO capitalized on the effectiveness of in-country volunteer efforts and the simpler-to-implement short-term educational schemes to track EQA performance before and after a 12–18-month period of active feedback on survey reports. This entailed site visits to each laboratory for initial assessment and training, continued follow-up by a PO volunteer during the active feedback phase, and the securing of external funding, which have proved to be limiting factors long-term.

Since 2019 to the present day, PO and RCPA continue to support 6 laboratories, with plans to enroll more in the coming 2 years. Despite challenges with inconsistent timely and complete result submission, often due to inconsistent access to reagents, supplies, service, and dedicated personnel and, more recently, the COVID-19 pandemic, a pattern of steadily improving EQA performance has been observed in this cohort.

Our current goal is to shift emphasis from costly and laborious on-site EQA training to virtual approaches that can reach out to a wider audience. Annual training workshops in one regionally accessible location may then supplement these courses. In this as in other endeavors, the support of volunteers from our network who are willing to engage with laboratories on an individual basis or small-group basis remains crucial for sustainable progress.

Discussion

It is undeniable that PALM services are critical to functional health systems globally; however, building sustainable capacity for these services in LMICs continues to be a challenge. One potential barrier to capacity building efforts is the fragmented and disease-specific nature of many global health programs. PO demonstrates that a network of dedicated volunteers, in partnership with corporations and professional and non-profit organizations, can initiate sustainable change in the quality of PALM services in LMICs by focusing their combined efforts on the three pillars of laboratory quality: human resources, systems, and quality and accreditation.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

AN collected the information featured in **Table 1**. All authors contributed to the conception, drafted components, and approve of the final version of the work submitted.

Acknowledgments

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Conflict of interest

TA was employed by ICON Laboratory services LLC. AN was the owner of Impala Consulting PLLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Informing healthcare operations with integrated pathology, clinical, and epidemiology data: Lessons from a single institution in Kenya during COVID-19 waves

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Pathology, clinical care teams, and public health experts often operate in silos. We hypothesized that large data sets from laboratories when integrated with other healthcare data can provide evidence that can be used to optimize planning for healthcare needs, often driven by health-seeking or delivery behavior. From the hospital information system, we extracted raw data from tests performed from 2019 to 2021, prescription drug usage, and admission patterns from pharmacy and nursing departments during the COVID-19 pandemic in Kenya (March 2020 to December 2021). Proportions and rates were calculated. Regression models were created, and a *t*-test for differences between means was applied for monthly or yearly clustered data compared to pre-COVID-19 data. Tests for malaria parasite, *Mycobacterium tuberculosis*, rifampicin resistance, blood group, blood count, and histology showed a statistically significant decrease in 2020, followed by a partial recovery in 2021. This pattern was attributed to restrictions implemented to control the spread of COVID-19. On the contrary, D-dimer, fibrinogen, CRP, and HbA1c showed a statistically significant increase (p-value <0.001). This pattern was attributed to increased utilization related to the clinical management of COVID-19. Prescription drug utilization revealed a non-linear relationship to the COVID-19 positivity rate. The results from this study reveal the expected scenario in the event of similar outbreaks. They also reveal the need for increased efforts at diabetes and cancer screening, follow-up of HIV, and tuberculosis patients. To realize a broader healthcare impact, pathology departments in Africa should invest in integrated data analytics, for non-communicable diseases as well.

KEYWORDS

integrated data, pathology, pharmacy, laboratory, nursing, epidemiology, COVID-19, Kenya

Introduction

Healthcare demands and the infrastructure required to meet those demands have become increasingly complex in modern times. In fact, these have radically changed during the coronavirus disease 2019 (COVID-19) pandemic, especially during the peaks or surges. To date, over 535 million cases and over 6.3 million deaths have been recorded (The Johns Hopkins Coronavirus Resource Center[1] [Accessed 13 June 2022]). In Africa, although it initially appeared that the disease had an attenuated course in terms of cases and mortality, the impact is by no means insignificant (1–4). Since the definition of the first COVID-19 case in Kenya, March 2020, five waves have occurred, resulting in over 5,600 deaths (Ministry of Health, Republic of Kenya[2] [Accessed 13 June 2022]). Similar to global observations, the implications of these surges have been a massive influx of COVID-19 patients, some of whom required intensive care, marked pressure on pathology services, delivery of nursing care, supply of therapeutics, excess mortality, and a plethora of downstream healthcare needs generated by the pandemic (5–8).

From a pathology perspective, the sheer number of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) tests, in the form of reverse transcription polymerase chain reaction (RT-PCR), in combination with all the logistical complexities of high volume sample and reagent management, introduced enormous pressure in laboratories. Important to note that, in low- and middle-income countries (LMICs), particularly in Africa, only a few laboratories had existing molecular diagnostic capacity, and hence, these had to be developed in record time to meet the clinical demand for testing (9, 10). There was also a ripple effect in all disciplines of pathology; clinical chemistry, microbiology, hematology, and anatomic pathology. A South African study looking at the short-term effects of test ordering coming from routine follow-up of patients with communicable and non-communicable diseases (NCDs) noted a significant drop (up to 80% for some tests), in orders for creatinine, lipids, HbA1c, thyroid-stimulating hormone (TSH), and free triiodothyronine (fT3), as a consequence of lockdown (11). Another study observed increased ordering of procalcitonin and lactate dehydrogenase, driven by a clinical demand for prognostication purposes. On the contrary, volumes in hematology and virology declined (12). In South Australia, a decline in almost all pathology tests except for molecular microbiology was noted. In particular, troponin, a marker of acute coronary syndrome (ACS) care, declined, begging the question of whether this was caused by a reduced incidence of ACS or because of hospital avoidance (13).

Prescription drug utilization also changed during the pandemic, and a particular note has been made of antibiotics: β-lactams, macrolides, fluoroquinolones, glucocorticoids; dexamethasone, prednisolone, and methylprednisolone were used especially for moderate and severe COVID-19 (14). Newly designed or repurposed therapeutics to treat severely ill COVID-19 patients were quickly adopted or as soon as clinical trials reporting variable degrees of efficacy were reported: monoclonal antibodies; SARS-CoV-2 neutralizing types, and anti-inflammatory types such as tocilizumab, an anti-interleukin-6 (IL-6) receptor antibody, and remdesivir, an antiviral agent (15, 16). In Kenya, remdesivir became available around June 2020. Tocilizumab had been in use for the treatment of severe rheumatoid arthritis; its use, however, increased significantly with the pandemic, perhaps also because our institution was a study site for the EMPACTA trial which evaluated the efficacy of tocilizumab in hospitalized patients with COVID-19 pneumonia (17).

Closely related to therapeutics was the unmet need for in-patient care. This includes not just intensive care unit (ICU) beds but also increased demand for intensivists, nursing care, and personal protective equipment (7, 18, 19). And, all this in a background of other communicable and NCDs, medical and surgical healthcare needs, that now had to be conducted with heightened infection control protocols. In this volatile public health climate, data and indicators related to the pandemic such as positivity rate, incidence rates, clinical trials hospitalization rates, case-fatality ratio, and vaccination coverage published in publicly available national or global platforms proved to be extremely useful for both patient treatment and policy or guideline making.

Having experienced numerous healthcare delivery challenges caused by the pandemic, we sought to identify evidence-based tools that may be useful for healthcare planning at an institutional level. We become aware that, although a large amount of data has been captured in the institutional healthcare information systems, analysis of these data for evidence-based planning was lagging. Pathology and epidemiology data appeared to be central; however, an integrated approach encompassing data from clinical care teams, pharmacies, and therapeutics promised greater insights. In addition, guidelines or policies based on global data required contextualizing using local data for meaningful local interventions, not to mention that institutional and local data are tributaries of global data. Given that any policy or guideline has to be changed constantly with the evolution of the pandemic and that they have significant public health and resource ramifications, reliance on robust and accurate data is crucial. The aim of this study, therefore, was to demonstrate how departments of pathology and clinical care teams can play a broader healthcare role by analyzing and describing the patterns of utilization of healthcare variables. Although the focus of the study was on COVID-19, the approach is applicable to other health problems for which we need to understand the interaction of science,

1 https://coronavirus.jhu.edu/map.html

2 https://www.health.go.ke/

policy, socioeconomic factors, and health-seeking and delivery behavior in Africa.

Materials and methods

Study setting and design

This was a cross-sectional, multidisciplinary study including the departments of Pathology, Nursing, and Pharmacy, at the Aga Khan University Hospital, a 280 bed tertiary, teaching, and referral hospital in Nairobi, Kenya. A temporary field hospital was set up to increase the capacity for taking care of COVID-19 patients, and this brought the bed capacity for COVID-19 patients to 88, of which 11 were ICU beds. Our objective was to explore data analytics for describing the patterns of healthcare utilization using indicators from the laboratory, pharmacy, and clinical care teams in an integrated fashion. The rationale for the study was that such utilization patterns would not only feed into larger national and international data sets that are used for modeling and projections but would also be critical in planning for near and intermediate future healthcare needs. Although the focus was on data around COVID-19, the bigger picture we envisioned was that African countries would use a similar approach to develop additional tools for a more efficient allocation of scarce health resources and improved healthcare operations. Given that many health problems, from seasonal infections to cancer and to metabolic diseases, that come to the attention of healthcare workers be they physicians or nurses will require a laboratory test and/or prescription, a steady source of data is guaranteed.

Data retrieval

We began by developing a list of both laboratory tests and prescription drugs whose turnover was perceived or projected to change as the pandemic evolved. These included those that were flagged for stock outs, on the one extreme, or as slow-moving on the other. From the existing hospital information system, queries to extract raw data were generated. The time frame was March 2019 to December 2021, with 2019 pre-pandemic data serving as a baseline, and 2020 to 2021 as 2 years of ongoing COVID-19 pandemic data. Laboratory data retrieved were monthly clustered tallies for tests including malaria parasite, D-dimer, fibrinogen, C-reactive protein (CRP), procalcitonin, serum sodium, HbA1C, HIV viral load, blood group, blood count, cervical smear (PAP smear), histology, blood culture, Mycobacterium tuberculosis (MTB), rifampicin (RIF) resistance (MTB/RIF), SARS-CoV-2, RT-PCR, and summation of all laboratory tests. The list of tests was representative of all sections of the laboratory: clinical chemistry, hematology, blood bank, microbiology, molecular, and histology. Similarly, from the pharmacy, monthly clustered tallies of issued prescriptions for azithromycin, dexamethasone, tocilizumab, enoxaparin, fentanyl, remdesivir, and piperacillin/tazobactam (PIPZO) were retrieved. These drugs were selected based on the perceived changes that would occur in their utilization. As highlighted in the introduction, dexamethasone, remdesivir, and other anti-inflammatory agents were variably adopted for the management of severe COVID-19. In addition, we were also keen on knowing what the trends of antibiotic utilization would be, and therefore, we included azithromycin and PIPZO in our analysis. We did not delve into the area of rational antibiotic use in this paper. Pharmacy data were retrievable from 2020, and, hence for baseline, data for 3 months prior to the full-blown pandemic in the country (January to March 2020) were used. From the nursing department, we obtained bed occupancy for COVID-19 patients including the intensive care unit (ICU).

Data analysis

Laboratory data were in the form of raw numbers of each test performed and were clustered in monthly tallies. The institutional COVID-19 positivity rate was continuously monitored as a 7-day rolling positivity rate. In this manuscript, this was collapsed into a monthly rolling positivity rate. Pharmacy data were also collected as monthly tallies; however, due to the multiple dosage formulations available for each drug, standardized units were created. As an example, dexamethasone may exist in 2, 4, or 6 mg formulations, and to standardize, all issued doses were converted to 6 mg units. The standardized units for the other prescription drugs were as follows: enoxaparin, 80 mg; azithromycin, 500 mg; remdesivir, 100 mg; tocilizumab, 80 mg; PIPZ0, 4.5 g; fentanyl, 100 mcg. From the clinical care teams, daily censuses for in-patient and ICU COVID-19 patients were collected and monthly averages were calculated. Data analysis and visualization were performed using Microsoft Excel version 16 (Microsoft Corporation) and RStudio, running R software for data analysis, version 4.1.2 (Boston, MA). The monthly mean number of tests for each of the selected tests in 2020 and 2021 was compared to the corresponding baseline mean (2019 data). To determine the statistical difference in the means, a t-test (unpaired and unequal variance) was calculated, and p-values < 0.05 were considered to be statistically significant. With regard to prescription drugs, the relationship between the number of units for each drug was plotted against the COVID-19 positivity rate, and non-linear regression models were created. Best fit models were determined using visual inspection and partial F-test compared to the linear model, whereby p-values > 0.05 were considered to significantly improve model fit. Finally, the COVID-19 positivity rate was plotted superimposed over percentage ICU hospitalization trends, selected laboratory tests, and prescription drug utilization.

Ethics consideration

This study was approved by the Institutional Ethics Review Committee of Aga Khan University Hospital, Nairobi [2021/IERC-87(v2)]. The study only looked at institutional data sets from the health and laboratory information system. Patient information, charts, records, or samples were not the subject of this study, nor were they investigated.

Results

By December 2021, close to 118,000 SARS-CoV-2 PCR tests had been performed. Five distinct waves with the zenith points in the months of July 2020, November 2020, March 2021, August 2021, and December 2021 occurred as shown in Figure 1. The characteristics of this trend matched very well with the national tracking which contained a larger sample size, giving external validity to the data set. Genomic epidemiology studies in the country revealed that the first two waves were predominantly caused by the original SARS-CoV-2 strain, while the third, fourth, and fifth were caused by the *Alpha*, *Delta,* and *Omicron* variants, respectively (20). The trend of the percentage of patients in ICU generally followed the positivity rate with less accentuated peaks. The initial ICU proportion in March and April 2020 was high (33%). This was thought to be due to the relatively small number of COVID-19 patients at the beginning of the pandemic, as opposed to overrunning of the bed capacity.

Analysis of laboratory tests revealed two major groups. The first was those whose test volumes decreased in the first year (2020) and then rose in the following year. Some recovered only marginally, but others to a level near the baseline, or in a few cases, surpassing the baseline. Included in this group malaria parasite test, MTB/RIF, blood group, blood count, and histology showed a statistically significant decrease. Blood count, a test included in diagnostic investigations, decreased by 15%, an average of 1,814 tests (95%CI: 397.2–3229.6; *p*-value 0.016). This was followed by only 6% growth for this test in 2021 compared to 2019 which was not statistically significant. On the contrary, the monthly average tests for malaria in 2020 decreased by 480 tests (95%CI: 218.9–740.4; *p*-value 0.001), translating to a 40.2% decline from 2019. This decrease persisted and was still statistically significantly lower than in 2019 (*p*-value <0.001). Other tests which showed a decrease, although not statistically significant, were serum sodium, PAP smear, blood culture, HIV viral load, and all laboratory tests summed together. The majority of these test orders showed variable recovery in 2021; however, test numbers for malaria and blood groups showed negligible recovery. The immediate explanation for this group was the restrictions implemented to control the spread of COVID-19 resulting in hospital avoidance. The second group was those whose test volumes increased despite restrictions both in the first and second year of the pandemic. These were thought to be caused by an increased utilization related to the clinical management of COVID-19. Included in this group were D-dimer, fibrinogen, CRP, and HbA1c. Tests for D-dimer, for instance, increased by more than four times from 2019 to 2021, an average of 114 to 525 (*p*-value <0.001). Orders for HbA1c initially showed a modest increment in 2020, but this rose by an average of 428 tests (95%CI: 346.6–508.2; *p*-value <0.001), translating to a 38.4% increment. Figure 2 and Table 1 show the graphical visualization and statistical analysis of the changes in the monthly test volume means in 2020 and 2021 compared to the baseline (2019).

Pharmacy data also revealed a gradual to sharply increased utilization of the selected prescription drugs. As visualized in Figure 1, the utilization of dexamethasone, tocilizumab, and remdesivir peaked during the third (*Alpha* variant) and fourth (*Delta* variant) waves that saw positivity rates of 28.1 and 16.8%, respectively. Although there was an increase in utilization during the *Omicron* variant wave, which had the highest positivity rate (42.6%), it was still less compared to the previous two waves. The models for prescription drug utilization during the 2 years followed a non-linear regression model in relation to the positivity rate as shown in Figure 3. Oral dexamethasone, tocilizumab, enoxaparin, azithromycin, and remdesivir best fit a quadratic model (polynomial regression, degree four). The models were better than the linear models, with a partial F-test showing significant *p*-values ranging from 0.045 in the case of remdesivir to <0.0.001 in the case of enoxaparin, and the R^2 values ranged from 0.31 in the case of PIPZO to 0.72 in the case of azithromycin. The regression for PIPZO best fit a cubic model; however, this was not any better than the linear model (*p*-value 0.6). Fentanyl (model not shown) best fit a quadratic model but was only marginally significant (*p*-value 0.036). The model for intravenous dexamethasone, unlike oral dexamethasone, was only marginally significant (*p*-value 0.047) on a cubic model (model not shown). In this study, we did not distinguish usual-care thromboprophylaxis from therapeutic-dose anticoagulation in regard to enoxaparin. The antibiotic PIPZO, typically used in the ICU setup, remdesivir, fentanyl, an anesthetic adjunct, showed weak predictive models. Tocilizumab, whose utilization was low in pre-COVID-19 times, increased with rising cases. A common feature noted for the pharmacologic agents was that the utilization was highest between March and August 2021, when *Alpha* and *Delta* were the predominant circulating variants. By the end of December 2021 which was the cutoff for our data collection, the *Omicron* wave was still ongoing and was the highest in terms of the number of cases and positivity rate. This was, however, characterized by a markedly reduced utilization, compared to the previous waves. The impact of the *Omicron* variant wave may therefore not be fully evident.

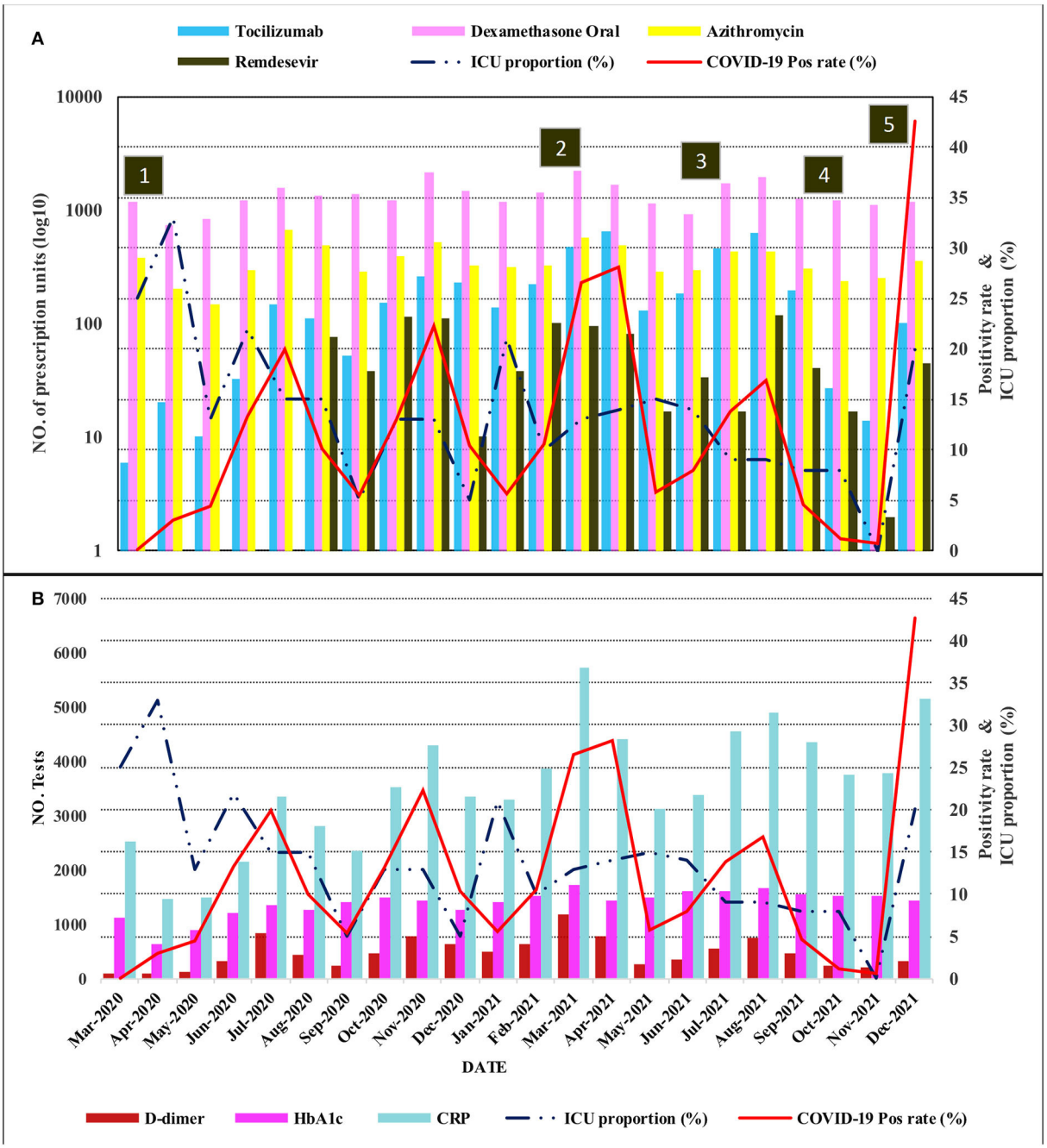

FIGURE 1
Compound plot showing the trends in the positivity rate and proportion of patients in ICU as line graphs on the right vertical axes. The movement prescription drugs and laboratory tests are plotted on the left vertical axes using bar charts in **(A,B)**, respectively. The prescription drug scale is log10 transformed. The horizontal axis (time series from March 2020 to December 2021) is common for the vertically aligned plots (ICU, intensive care unit; CRP, C-reactive protein). **Annotation in numbered squares**: (1) Impositions of restrictions and curfew, (2) start of vaccination and rise of predominantly *Alpha* variant, (3) *Delta* variant wave, (4) Lifting of restrictions and curfew, (5) *Omicron* variant wave.

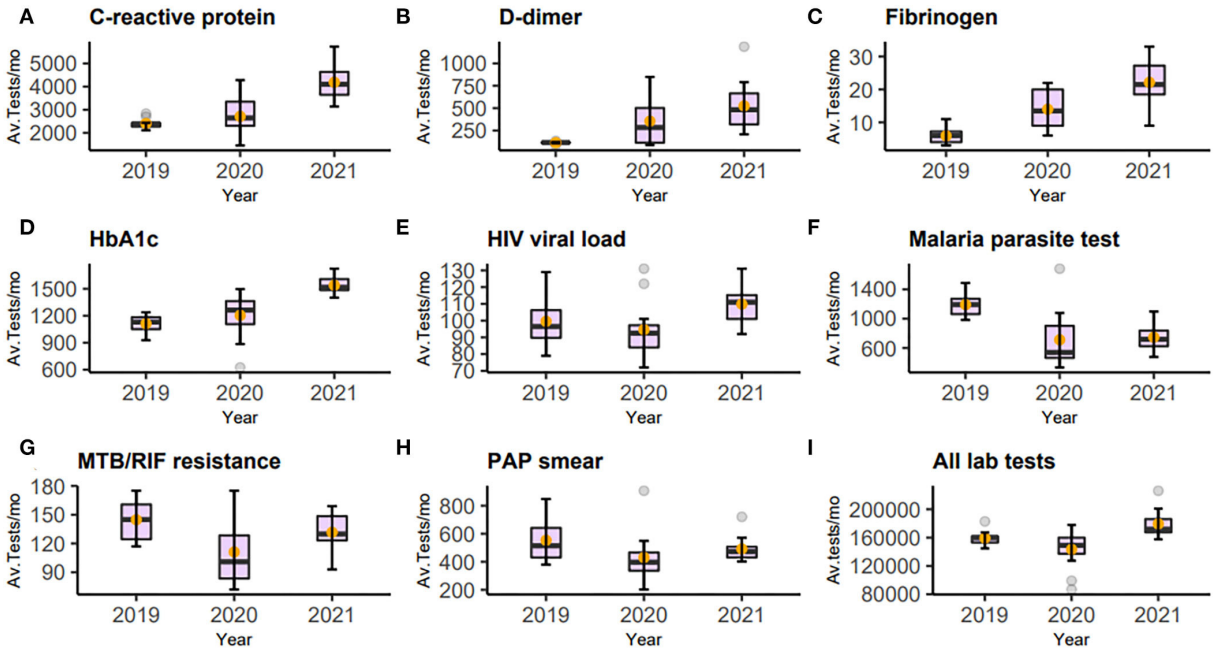

FIGURE 2
Figure of box-and-whisker plot **(A–I)** representing C-reactive protein, D-dimer, fibrinogen, HBA1c, HIV viral load, Malaria parasite test, MTB/RIF, PAP smear, and all laboratory tests. Each panel shows the changes in average tests per month (Av. Tests/mo) from 2019 to 2021 The mean is represented by the orange dot. The statistical summaries of these and other tests not plotted are shown in Table 1.

Discussion

Laboratories regularly use data on test volumes and results to determine among other laboratory operations, reagent management, and workflow. The same is true for other departments in a healthcare facility whose aims are to meet the demands of testing, diagnosis, treatment, or prevention. These data, if carefully analyzed, may, however, be utilized for optimizing healthcare operations at a public health level. In this study, we have demonstrated that there was significant upward demand for CRP, fibrinogen, D-dimer, and HBA1c test in relation to rising cases and care of COVID-19 patients. Other laboratory tests were variably affected potentially due to social–economic factors. The demand for COVID-19-related therapeutics was best predicted by quadratic regression models. The highest demand for both tests and therapeutics was recorded during the *Alpha* and *Delta* variant waves of the pandemic.

One finding that has also been observed by other investigators was that interventions influenced by social political and public health concerns determined health-seeking behavior during the pandemic. Various degrees of restrictions and lockdowns likely lead to loss of income, hospital avoidance, fear of contracting COVID-19, postponement of elective surgical cases, and restricted travel (8, 21). In our study, the pattern for the malaria parasite test was perhaps an example where restricted movement changed testing demand and, by extrapolation, disease epidemiology. Nairobi, which is the county the study was conducted in, is not endemic to malaria (22). Typically, people contract malaria when they travel to malaria endemic counties, and therefore, a restricted movement was likely to lead to the further reduced incidence in Nairobi and hence reduced testing requirements. This observation may not be true for malaria endemic regions and for other diseases endemic in distinct geographic distributions.

There is no doubt that social restrictions were beneficial in managing COVID-19 patient numbers, especially until other interventions, such as vaccines, were widely available. However, one can see the potential negative effect of loss of continuity in care due to prolonged restrictions, for example, diseases such as tuberculosis (TB) and HIV whose prevalence in Africa is still high. Initial diagnosis and follow-up rely heavily on MTB/RIF and HIV viral load testing, and any lapse in the diagnosis, treatment, or follow-up would lead to loss of the benefits accumulated by the TB and HIV control programs (23). A study conducted in Nairobi, Kenya, implemented real-time monthly surveillance of TB and HIV activities to counteract the feared negative impact on TB and HIV programs. Small successes in treatment, follow-up, and referral were registered, showing the usefulness of more active intervention (24). This

TABLE 1 Table showing analysis of the changes in laboratory test in 2020 and 2021 compared to 2019 data.

	2019	2020			2021		
Test	**Monthly mean (Range)**	**Mean (Range)**	**Δ Mean [CI 95%]**	**t-statistic (*P*-value)**	**Mean (Range)**	**Δ Mean [CI 95%]**	**t-statistic (*P*-value)**
D-dimer	114 (83–135)	355.8 (89–849)	+240.8 [65.3–416.3]	3.02 (0.01)	525 (207–1187)	+411 [229.6–592.4]	4.98 (<0.001)
Fibrinogen	5.9 (3–11)	14 (6–22)	+8.1 [4.4–11.8]	4.64 (<0.001)	22.2 (9–33)	+16.3 [11.4–21.1]	7.16 (<0.001)
Malaria	1192.3 (983–1489)	712.7 (334–1,683)	−479.6 [218.9–740.4]	3.93 (0.001)	747.3 (478–1,098)	−445 [301.1–589.1]	6.41 (<0.001)
C-reactive protein	2401.8 (2,113–2,829)	2,712 (1,453–4,281)	+310.2 [0–225.2]	1.26 (0.23)	4186.9 (3,136–5,727)	+1785.1 [1268.3–2,302]	7.5 (<0.001)
HbA1C	1111.6 (928–1,239)	1204.1 (624–1,497)	+92.5 [0–72.2]	1.20 (0.25)	1,539 (1,401–1,723)	+427.4 [346.6–508.2]	10.97 (<0.001)
Cervical smear	553.6 (380–848)	430.6 (202–907)	−123 [0–262.5]	1.83 (0.08)	492.3 (402–721)	−61.3 [0–165.4]	1.24 (0.23)
MTB/RIF	144.8 (117–175)	111.2 (72–175)	−33.6 [6.6–60.6]	2.62 (0.02)	131.7 (93–159)	−13.1 [0–31.2]	1.49 (0.14)
HIV viral load	99.6 (79–129)	94.6 (72–131)	−5 [0–19.3]	0.72 (0.48)	109.8 (92–131)	+10.2 [0–2.2]	1.71 0.10
Serum Sodium	5242.3 (4,849–5,813)	4712.8 (2,339–5,631)	−529.5 [0–1,060]	2.15 (0.05)	5607.9 (5,104–6,587)	+365.6 [38.7–692.4]	2.36 (0.030)
Procalcitonin	736.4 (84–1,071)	733.8 (397–1,025)	−2.6 [0–185.2]	0.03 (0.98)	916.9 (616–1,289)	+180.5 [0–16.4]	1.90 0.07
Blood group	388 (333–441)	324.5 (229–520)	−63.5 [15.4–111.6]	2.83 (0.013)	353.8 (315–387)	−34.2 [11.9–56.4]	3.19 (0.004)
Blood count	11717.8 (10,660–14,100)	9904.3 (6,247–13,098)	−1813.5 [397.2–3229.6]	2.74 (0.016)	12479.5 (10,448–16,766)	+761.7 [0–445.6]	1.34 (0.19)
Blood culture	457.8 (428–518)	415.5 (279–578)	−42.3 [0–95.5]	1.71 (0.11)	484.1 (426–621)	+26.3 [0–13.2]	1.41 (0.17)
Histology	1407.9 (1,173–1,668)	1254.3 (785–1,450)	−153.6 [10–296.8]	2.23 (0.037)	1374.7 (1,179–1,658)	−33.2 [0–157.5]	0.55 (0.58)
All lab tests	159,385 (144,776–182,872)	143416.3 (86,696–177,800)	−15968.8 [0–34,165.3]	1.89 (0.08)	179385.2 (157,600–226,159)	+20000.2 [6945.2–33055.1]	3.24 (0.005)

The monthly mean test numbers and range for each year are shown. The differences in mean (Δ Mean) have been calculated, and increase or decrease compared to 2019 is depicted using (+) or (–) symbols, respectively. To test for statistical significance in the change, unpaired t-test, assuming unequal variance was applied. The t-statistic, corresponding 95% confidence interval (CI 95%), and p-values are presented. The last row (All lab tests) represents the monthly mean number of all tests performed in the laboratory including those not analyzed individually.

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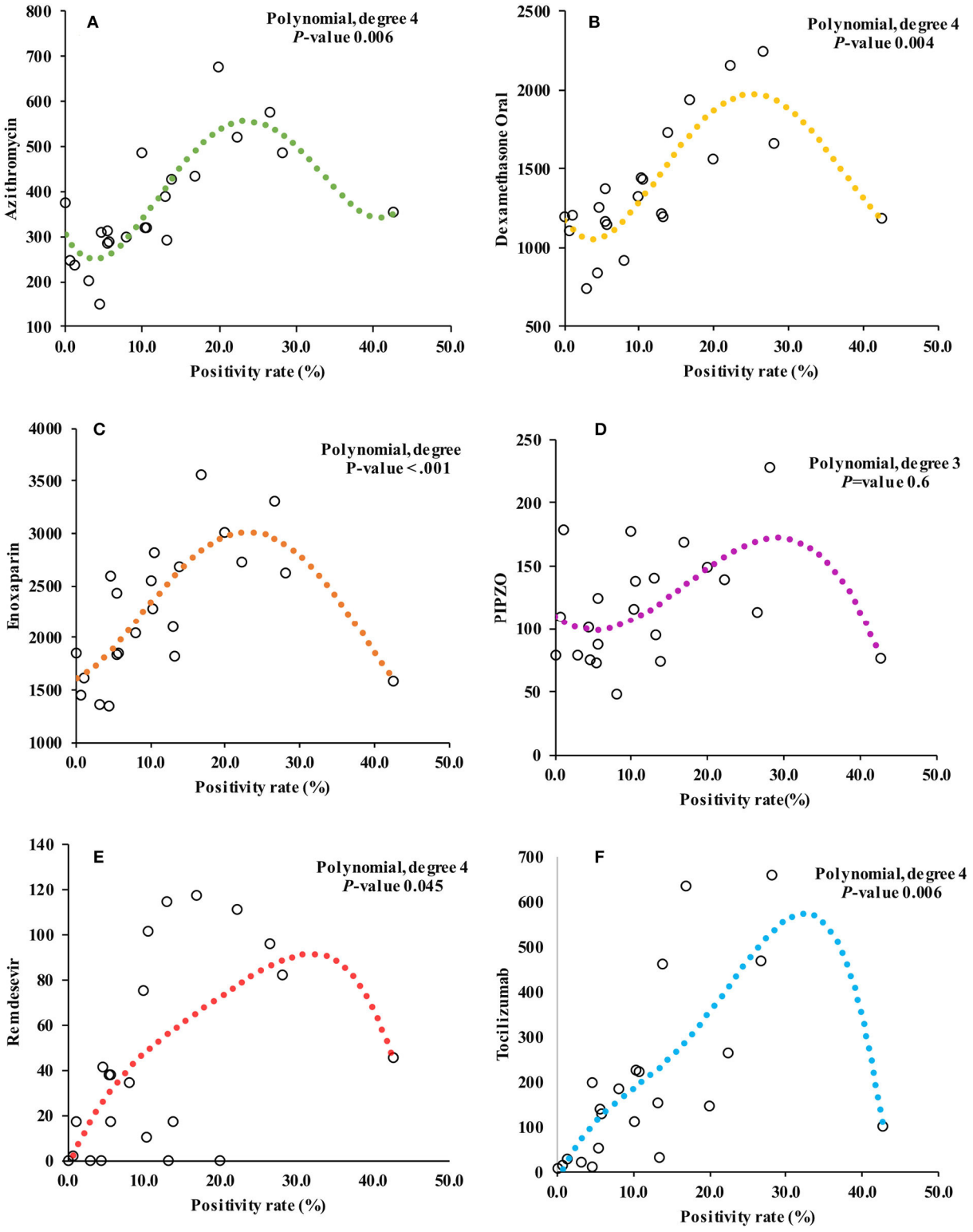

FIGURE 3
Figure showing the relationship between positivity rate and monthly prescription drug utilization. **(A–F)** represent the models for azithromycin, oral dexamethasone, enoxaparin, piperacillin/tazobactam (PIPZO), remdesivir, and tocilizumab, respectively. The models are non-linear, and polynomial regression best fitted a quadratic model with the exception of PIPZO, **(D)** which best fitted a cubic model. The *P*-values for each model shown are the outcome of partial F-test to determine whether there was a statistically significant difference from the linear model. The model for PIPZO was not significant. Not shown, are models for fentanyl (quadratic model, *p*-value 0.03) and intravenous dexamethasone (cubic model, *p*-value 0.04).

trend and potential intervention may also apply to cervical cancer screening which is dependent on PAP smears and human papillomavirus (HPV) testing. In the majority of African countries, screening uptake is already suboptimal, and this is in addition to East Africa having the highest cancer of the cervix disease burden (25). Loss of screening momentum is therefore a drawback in reducing the incidence of invasive cancer of the cervix. A late-stage cancer diagnosis is also a recurring health problem but is likely more common in Africa (26, 27). Therefore, decreased histology services could potentially mean delayed surgeries and diagnosis including cancer diagnosis and a worsening of late the stage cancer diagnosis problem.

Another pattern of interest was HbA1c testing. Testing for HbA1c is typically performed as a diagnostic test for diabetes or to monitor glycemic control over the last 3 months. Increment in HbA1c testing appears to be intuitive, given the fact that diabetes is a leading risk factor for severe COVID-19. However, the rapid rise points to COVID-19 unmasking a significant population with undiagnosed diabetes in the community. In a recent publication from our institution, including 913 records of admitted patients, the proportion of COVID-19 patients with diabetes at baseline was 27.3% and this rose by over 20% over the course of admission, bringing the proportion with diabetes to 48.1% (28). Given that glucocorticoids are essential for hospitalized patients with COVID-19, pre-diabetics who were tipped over to overt diabetes may have contributed to this proportion.

The interaction between pathology, pharmacy, and clinical care needs clearly emerged in our analysis. Hemostasis appears to have been a major issue given the simultaneous rise in prescriptions for enoxaparin and increased demand for fibrinogen and D-dimer test especially when hospitalization was high. Inflammation and immune dysregulation also were critical areas given the concurrent rise in inflammatory marker testing (CRP and procalcitonin) and inflammation modifying agents (dexamethasone and tocilizumab). Notable was the relatively modest increase in the utilization of prescription drugs during the *Omicron* variant wave which was the highest in terms of positivity rate. This provided some evidence that the *Omicron* variant was associated with less severe disease than the previous variants, with contributing factors being vaccination and immunity acquired *via* natural infection (29, 30). This observation should, however, be interpreted with the caveat that the impact of the *Omicron* variant may not have fully emerged since this wave was still ongoing at the cut-off time for our data collection. At an operations level, this pattern of decreasing utilization means that the demand for drugs used for COVID-19 patients and tests pushed up by COVID-19-related testing would decrease. Rationally, one would commensurately decrease procurement to avoid the other extreme problem of overstocking and expiry leading to healthcare resource wastage.

The study was limited by the lack of patient-level integration and analysis of the results of tests included in this study. The trends of test positive MTB/RIF, malaria, or out-of-range HbA1c, for instance, would provide more granular data on the translation of diagnostics to clinical care and treatment. In addition, data on which patients had what test, for what diseases, and what treatment was given would provide key information on the extent to which healthcare needs are met by a particular program, assess adherence to good clinical practice, and facilitate national healthcare planning. Furthermore, this was a targeted analysis; we only looked into a small subset of pathology, pharmacy, or nursing outputs. A comprehensive and unbiased analysis would, of course, require more sophisticated computational capacity. Although this was a targeted, single-institution study, we assume that we have demonstrated the power of integrated data analysis would have, when applied at a national level for the purposes of healthcare planning in Africa.

Data analytics has become a major tool in the study of global social and economic matters. Laboratories produce vast amounts of data; unfortunately, most of these data lie unanalyzed and therefore unusable by the community. These data are not only important for the estimation of disease prevalence but can also be indicators of access to treatment, follow-up, and unmet clinical needs. For example, during the first two waves, stock outs of laboratory reagents and consumables disrupted operations and by extension clinical care. This was occasioned by the abrupt changes in demand for tests outlined above combined with the need to process large numbers of COVID-19 tests. We, therefore, utilized the data we had collected, and the national epidemiological projections to inform the projected third wave. This prompted a targeted stocking of essential drugs, laboratory, reagents, and consumables resulting in better preparedness, a significant reduction in stock outs, and reduced disruptions in testing and healthcare delivery in the subsequent waves.

The results of this study inform us of the expected scenario in the event of other outbreaks with similar pathophysiology as COVID-19. Furthermore, they lead us to recommend increased efforts at diabetes screening, cervical cancer screening, and cancer screening as well as active follow-up of HIV and TB patients who may have discontinued follow-up. Harnessing data analytics is probably as important as investing in technology and human resource to improve pathology in Africa. With regard to future prospects, the wealth of information from this limited analysis makes a strong case for expanding its scope. The data that we have collected and analyzed will be an excellent resource for pathology and other clinical departments since other health data analytical projects can plug into this base, and the process can be amplified by multicenter partnerships. This will also be a stepping stone to "big data" analysis which is promising enormous potential even in healthcare (31). Investing in data

information systems that can be seamlessly interlinked with other clinical public health departments, real-time analytics, and contributing to policy formulation will be a double-edged sword that would enable pathology departments to exert a broader impact in public healthcare.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

Author contributions

AN, JK, JG, and DN: conceptualization. AN: retrieval pathology data, data analysis, visualization, and manuscript writing. JK and DN: retrieval and clean-up and analysis of hospitalization data. JG: retrieval and clean up and analysis of pharmacy data. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.969640/full#supplementary-material

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Genomic characterization of two community-acquired methicillin-resistant *Staphylococcus aureus* with novel sequence types in Kenya

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Staphylococcus aureus is a clinically important bacteria with high antimicrobial resistance (AMR) challenge globally. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) clones with unique sequence types have been identified in the community showing evidence that the epidemiology of MRSA globally is changing and requires continual surveillance. We utilized whole genome sequencing to characterize two community acquired-MRSA (CA-MRSA) strains isolated from wound swabs from community-onset infections in two health facilities in Kenya. The two strains belonged to multilocus sequence type (MLST) sequence type (ST) 7460, and ST 7635. The resistance genes detected showed that the novel STs are carriers of clinically relevant resistance genes. Linezolid and mupirocin resistance was observed, yet mupirocin is not commonly used in the country. Mutations within resistance genes were also detected and the pathogenicity toward the human host matched various pathogenic global *S. aureus* families, e.g., *S. aureus* subsp. *aureus* USA300. Multidrug efflux transporters, important in antimicrobial resistance including restriction enzymes type I and type IV were detected. Plasmids identified showed similarities with the plasmids in other clinically significant non-staphylococcal species, such as *Pseudomonas aeruginosa, Escherichia coli, Morganella morganii,* and *Enterococcus faecium*. Both STs belong to clonal complex 8 (CC8) which is the most successful MRSA clone in Kenya. Spa type t30 to which ST 7635 belongs has not been reported in the country. The results of this study further highlight the need for epidemiological studies to reveal circulating strains and antimicrobial resistance spread between hospitals and the community. The genomic research highlights resistance to anti-staphylococcal broad-spectrum antimicrobials not used frequently in the

country, jeopardizing successful MRSA treatment since most health facilities do not perform genotypic resistance tests for routine patient management. Preliminary insights into unidentified STs of CA-MRSA in Kenya show the need for molecular epidemiological surveillance studies to further understand the diversity of *S. aureus* in Africa.

KEYWORDS

CA-MRSA, WGS, novel sequence, AMR, Kenya

Introduction

Staphylococcus aureus (*S. aureus*), a commensal in humans and an abundant bacteria of the skin microbiome, is a major pathogen that causes a wide range of clinical infections (1, 2). *S. aureus* has thrived as a human pathogen due to its ability to infect any tissue, for example, the skin, where it adapts within the environment and transitions to cause infection (3). The growing prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) presents a clinical challenge in the management of serious infections worldwide (4).

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) clones with unique sequence types have been identified in the community, suggesting evolution within *S. aureus* (5). The epidemiology of MRSA worldwide is changing, and a rise in strains causing disease in populations with no associated risk factors in the community has been reported (6, 7). CA-MRSA has in the past two decades emerged as a clinically relevant pathogen causing skin and soft tissue infection (SSTI), which is cytotoxin driven with suggestions indicating that hypervirulent sequence types (STs) exist (7). MRSA has evolved over the years, from which clinically significant STs reported range from the 1960s. A good example is the ST250 MRSA-I published around 1965, consistent with ancient European types and the most widespread multidrug-resistant clone globally. ST1-MRSA-IV was reported in Australia in 1981 and the United States in 1990, and it is known to have high-level mupirocin resistance and multi-drug resistance characteristics. Novel strain ST2249-MRSA-III which was a multi-resistant clone was reported in 1973 and was important because gentamicin resistance first appeared in this strain. It is believed that 35.3% of ST239 chromosomes were inherited by this strain, and it's also known to have caused the Australian epidemic in the early 1970s (8).

Community acquired-MRSA (CA-MRSA) has diverse clones which are dispersed in different clonal complexes, but only a few are genetically related (9). However, in Africa, *S. aureus* has not been the focus of research in the past despite the rich diversity that could have a significant impact, especially when studying the epidemiology of *S. aureus* infections (10). Knowledge of clones is important in medical practice because it allows antibiotic resistance studies to be done on each one of them. Notably, the molecular epidemiology of CA-MRSA shows poorly regulated sales of antibiotics in pharmacies, as well as empirical treatment, in the absence of laboratory investigation, contributes to the development of new MRSA clones with increased resistance to antibiotics (11).

Differentiating MRSA using whole genome sequencing (WGS) is important to characterize strain diversity, and understand evolution within MRSA (12, 13). In Kenya, a developing country, various sequence types of both MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) from both hospitals and community (some being global MRSA strains), have been identified and they include; ST 22, ST8, ST39, ST1290, ST241, ST1, ST5, ST8, ST152 from Thika (Kiambu county), Kericho (Kericho county), Nairobi (Nairobi county) and Kisumu (Kisumu county) (14). This is important in studying evolution in MRSA especially the co-existence between CA-MRSA and hospital-associated MRSA (HA-MRSA) and the emergence of new resistance as a result of co-existence. It also is essential in studying key genomic mutations which have a public health significance and impact, such as new insertion sequences (IS) that affect virulence and pathogenicity toward a human host. For example, symptoms presented by patients infected with MRSA strain with genomic mutations, such as IS256 in USA500, whose outcome causes increased virulence and heightened pathogenicity and cytotoxicity, may present differently in a different geographical setting. The purpose of this study was to characterize the two novel ST 7460 and ST 7635 by WGS, to determine antimicrobial resistance profiles, spread, virulence characteristics, and genetic diversity. Molecular typing of the novel isolates by WGS has enabled track spread of MRSA by MLST and has further provided an in-depth insight into MRSA evolution. The two isolates were from community-onset staphylococcal infection, from Kiambu and Nyeri County health facilities, which are AMR surveillance sites in Kenya. Characterization of the novel sequence types ST 7460, and ST 7635, further highlight the need for medical laboratories to perform molecular diagnosis, including antibiotic resistance

gene detection and resistance to specific antibiotics, due to other mechanisms. Molecular diagnostic testing is infrequently performed in medical laboratories and less so in resource-limited countries. Performing molecular diagnosis will guide clinicians on how best to manage their patients.

Materials and methods

Study isolates

Two CA-MRSA isolates from a surveillance study of SSTIs in five AMR surveillance sites in Kenya were characterized by WGS and had novel STs. A cross-sectional study design was employed, and a simple random sampling method was used. The samples were collected from outpatient departments of the selected facilities, within one year. All recruited patients did not have a clinical history/procedure within the past year and were not on antibiotic treatment for the SSTI. Patients recruited had clinical features of wound infection which brought them to the clinics. They had no history of international travel (never traveled abroad). Bacteria isolation was done using blood agar and identification was performed using standard microbiology procedures (15), antimicrobial susceptibility was performed guided by the clinical laboratory standards institute (CLSI) (16) using Vitek Compact 2 (bioMerieux Inc), an automated bacteriology system that identifies the bacterial pathogen and tests for a panel of 20 antibiotics providing breakpoint susceptibility by providing 3 minimum inhibitory concentration (MIC) cut-off values at Resistance, Intermediate and Susceptible points.

Whole genome sequencing, genome assembly, and annotation

DNA library was prepared using the Collibri^{TM} PCR-free ES DNA Library Prep Kit (Thermofisher, Massachusetts, USA) and quantification was done using Collibri^{TM} Library Quantification Kit (Illumina, Inc., California, USA). Sequencing was performed on the Illumina MiSeq platform. Quality assessment for raw data was done by *fast QC* and data was trimmed using Trimmomatic (v 0.36) (17), and assembled using Shovill (v3.0) (18). QUAST (v 5.0) (19) and BUSCO (v 5.3.2) (20) were used to perform quality assessment for genome assemblies and annotations. SCC*mec*Finder and MLSTFinder, both hosted within the Centre for Genomic Epidemiology (CGE)[1] were used for SCC*mec* and MLST typing, respectively. Further, genome assemblies were run in PubMLST[2] (21) for Multi-locus sequence typing. Spa typing [Based on the sequence of the X polymorphic region of the A protein (spa) where the constitution of the X region is a variable number of 24-bp repeats flanked by well-conserved sequences/regions] was performed on the CGE platform using the Spatyper tool.[3] Further, the contig sequences were used to query for spa types on the web-based SpaTyper[4] and repeat sequences verified on the Ridom Spa Server[5] (22).

Resfinder and virulencefinder (both available at the center for genomic epidemiology CGE), were used to identify acquired antibiotic resistance/mutations within resistance genes and acquired virulence, respectively. PathogenFinder[6] was used to predict pathogenicity toward a human host. Mobile genetic elements (MGE) [7] based on top hits, were used to identify mobile genetic elements and their relation to antimicrobial resistance genes and virulence factors. Plasmid identification and homology parameters were set at 99–100%. A comprehensive antibiotic resistance database (CARD) [8] (23) was also used to identify resistance genes and resistance mechanisms. Assembled contigs were also submitted to the bacterial whole genome sequence database[9] (24) for whole genome sequence typing and source tracking. To add some epidemiological context to the novel STs we performed a phylogenetic analysis of 11 other reported MRSA strains from previous studies in the country (13, 25, 26). Concatenated sequences were retrieved from the PubMLST database and a multiple sequence alignment was performed using the Multiple Alignment with Fast Fourier Transform (MAFFT). A maximum likelihood tree with a generalized time-reversible model was constructed for the 12 local MRSA STs using the Molecular Evolutionary Genomic Analysis (MEGA) (25). Version 11.0. 11 software with bootstrapping parameter set at 1000. The tree was refined using the Interactive Tree of Life (iTOL v. 6.5.6) (**Figure 1**). The reference genome used was N315_BA000018_ST5.

Results

Characterization of isolates with novel alleles

Isolate SA004 contained a novel arcC allele number 757 and assigned novel ST 7460 and isolate SA002 with novel allele glpf number 936 and assigned novel ST 7635. The isolates were identified as belonging to spa type t1476 and t30, respectively.

1 https://cge.food.dtu.dk/services/
2 http://bacdb.cn/BacWGSTdb/
3 https://cge.food.dtu.dk/services/spaTyper/
4 https://spatyper.fortinbras.us/
5 https://spa.ridom.de/
6 https://cge.food.dtu.dk/services/PathogenFinder/
7 https://cge.food.dtu.dk/services/MobileElementFinder/
8 https://card.mcmaster.ca/analyze/rgi
9 https://bacdb.cn/BacWGSTdb

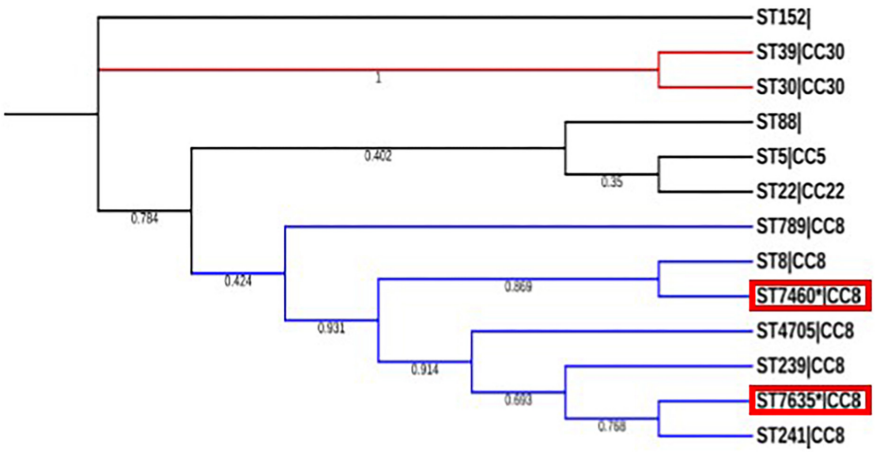

FIGURE 1
Genetic relatedness of the two novel sequence types to other sequence types identified in the country. The novel sequence types (ST7460 and ST7635) are highlighted in red boxes. The tree topology was based in 1000 iterations with bootstrap support expressed as probability values. The blue branches highlighted CC8 to which ST 7460 and ST 7635 belong.

The spa type of ST 7460 (t1476) and ST 7635 (t30) suggests that they may be members of the clonal complex 8 that constitutes ST8. Spa type t30 has not been reported in the country before. Interesting resistance was observed in the isolates, for instance, SA002 was only susceptible to 2 out of 13 antibiotics tested, while SA004 was only susceptible to 4 out of 13 antibiotics tested (**Table 1**). The antimicrobial susceptibility profiles of the two isolates with novel STs are in **Table 1**. SA002 was also found to be resistant to rifampin and linezolid phenotypically and had genes conferring rifampin resistance (*rpoB*), linezolid resistance (*cfr*), and mupirocin resistance (*mupA*) (**Table 2**). Various AMR gene families that are key to bacterial resistance were present in both SA002 and SA004 (**Table 3**). These include multidrug efflux transporters which are important in transporting antibiotics from inside to outside of bacterial cells (27–29) (**Table 4**).

Genomic characterization of the two isolates

WGS was used to characterize previously described AMR genes in both SA002 and SA004. Interestingly, restriction enzymes (**Table 4**) which are important for preventing gene transfer were detected (29). Both SA002 and SA004 had type I restriction enzymes which can block horizontal gene transfer between MRSA that are clinically important and type IV restriction enzymes which are important barriers to the transfer of plasmid DNA from other bacteria such as *Escherichia coli* (*E. coli*) (30). Important identified plasmids in SA002 were rep15 which carries resistance genes for tetracycline, clindamycin, trimethoprim, pristinamycin IIA, lincomycin, linezolid, chloramphenicol, florfenicol, tiamulin, mupirocin, ampicillin, cefoxitin, and gentamicin. The plasmid had an insertion sequence IS256 in the reverse strand and identity was 99.3% with an alignment coverage of 99.8%. Also, rep7a was detected and it carries resistance genes for tetracycline, lincomycin, clindamycin, pristinamycin IA, quinupristin, erythromycin, ampicillin, penicillin, and amoxicillin. The plasmid also carries virulence genes staphylokinase, gamma-hemolysin component B and C precursor, aureolysin, staphylococcal component inhibitor, and serine protease splA and B. The plasmid had an insertion sequence IS256 in the forward strand and identity was 100% with an alignment coverage of 100%. In SA004 important plasmids identified were rep10, which carries the resistance genes for trimethoprim, lincomycin, quinupristin, pristinamycin IA, erythromycin, penicillin, ampicillin, and amoxicillin. The plasmid also carries virulence genes staphylokinase and staphylococcal component inhibitor, and identity was 100% with an alignment coverage of 100%. Rep7a was also detected and it carries resistance genes for doxycycline, tetracycline, gentamicin, cefoxitin, ampicillin, and ampicillin + clavulanic acid. The plasmid also carries virulence genes leucocidin D component, gamma-hemolysin component B and C, aureolysin, serine protease splE, and identity was 100% with an alignment coverage of 100%. Both SA002 and SA004, based on top hits, had plasmids that showed similarity with plasmids identified in other clinically significant non-staphylococcal species and have also been detected in different geographical regions of the world such as pMP63C detected in *Morganella morganii*, pHVH-V1836-9 identified in *Enterococcus faecium* pIM13 identified in *Bacillus subtilis*, pK34-7-1 found in *Pseudomonas aeruginosa*, pK93G, and pT15G-1 found in *Staphylococcus lugdunensis*, The prediction of both isolates' pathogenicity toward a human host, with gene identification set at high-level similarity with database entries (95-100%), matched important *S. aureus spp.* such as

TABLE 1 Resistance profiles of SA002 and SA004.

ANTIBIOTIC/ ISOLATE	Cefoxitin	Penicillin	Erythromycin	Clindamycin	Ciprofloxacin	Tetracycline	Vancomycin	Tigecycline	Linezolid	Rifampin	Trimethoprim/Sulfamethoxazole	Mupirocin	Gentamycin
SA002	R	R	R	R	R	R	S	S	R	S	R	R	R
SA004	R	R	R	R	R	R	S	S	S	R	R	S	R

S stands for susceptible and R stands for resistant.

S. aureus subsp *aureus* USA300, known to be an epidemic clone of CA-MRSA, *S. aureus* subsp *aureus* str. Newman DNA, known to have robust virulence phenotypes, *S. aureus* subsp *aureus* NCTC 8325, known as the prototypical strain for genetic manipulation, *S. aureus* subsp *aureus* JH9, known to cause bacterial endocarditis and vancomycin-resistant *S aureus* infections (VRSA), *S. aureus* subsp *aureus* COL, known to cause MRSA infections, and *S. aureus* RF122, a major clone which causes severe bovine mastitis. The isolates also matched *S. aureus* strain MSSA476, known to have a protein msa (modulator of sarA) which enhances the expression of the staphylococcal accessory regulator (sarA) in a strain-dependent manner. sarA affects the transcription of accessory gene regulator (agr) and genes which encode virulence factors such as protein A (spa) and alpha toxin (hla). It also matched *S. aureus* subsp *aureus* JH1, known to cause bacterial endocarditis and MRSA infections, and *S. aureus* subsp *aureus* str. JKD6008 is known to cause both MRSA and VRSA infections.

Discussion

This is a WGS-based study where we characterized two unique CA-MRSA out of 65 *S. aureus*, collected from 5 AMR surveillance sites in Kenya, which had novel STs 7460 and 7635. Recruited patients had SSTIs, which were community-onset as defined by the Centers for Disease Control and Prevention (CDC) distinguishing criteria for CA-MRSA from HA-MRSA (31). MRSA remains very clonal in the country with four main clonal complexes (5, 8, 22, and 30) (**Figure 1**) isolated from only 5 counties in the country out of 47 counties. CC8 to which novel STs 7460 and 7635 belong is the most successful MRSA clone in Kenya. Previously reported MRSA sequence types in Kenya to include STs 5, 8, 22, 30, 88, 152, 239, 241, 789, and 4705 belonging to four main clonal complexes 5, 8, 22, and 30 showing evolution within the complexes as shown in **Figure 1**. This information is important in medical practice because it allows antibiotic resistance studies to be done, which can guide the use of empirical treatment and can also be used to identify the emergence of new MRSA clones with increased resistance to antibiotics.

Antimicrobial susceptibility tests showed that the novel CA-MRSA STs had phenotypic resistance to cefoxitin, penicillin, erythromycin, clindamycin, ciprofloxacin, trimethoprim/sulfamethoxazole, and tetracyclines. Interestingly, linezolid and rifampin resistance was observed in SA002. Genotypic characterization revealed that linezolid resistance gene *cfr*, mupirocin resistance gene *mupA*, and rifampin resistance gene *rpoB* were present in SA002. Rifampin resistance gene had mutations that conferred high-level resistance to rifampin, a trait common in nosocomial *S. aureus* infections, which shows the spread of resistance between

TABLE 2 Resistance genes and mutation points detected within SA002 and SA004.

Class	Resistance gene(s)	Mutations points	Genotypic resistance (SA004)	Genotypic resistance (SA002)	Phenotypic resistance (SA004)	Phenotypic resistance (SA002)
Tetracyclines	*tet(K), tet(M)*	–	+	+	+	+
Beta-lactams	*mecA, blaZ*	pbp2:p.A606D, pbp2:p.A420V, pbp4:p.P220S, pbp4:p.L234H, pbp2:p.A285P, pbp2:p.E315A, pbp4:p.L234H	+	+	+	+
Macrolides	*erm(C), erm(A)*	–	+	+	+	+
Quinolones	*gyrA, parC*	gyrA:p.D402E, gyrA:p.E859V, gyrA:p.V598I, grlA:p.V694M, grlB:p.D530G, grlB:p.E471K	+	+	+	+
Carboxylic acid	*mupA*	–	–	+	No MIC	No MIC
Aminoglycoside	*aac(6')-aph(2")*	–	+	+	+	+
Sulfonamides	*dfrG, dfrB*	dfrB:p.I97T, dfrB:p.V72E, dfrB:p.V72E	+	+	+	+
Oxazolidinone	*cfr*	–	–	+	–	+
Glycylcycline	–	–	–	–	–	–
Glycopeptides	–	–	–	–	–	–
Rifamycin	*rpoB*	rpoB:p.S529L, rpoB:p.G767S	–	+	–	+
Amphenicol	*cfr*	–	–	+	No MIC	No MIC

MIC stands for minimum inhibitory concentration and the symbol (+) represents present and (–) represents absent.

hospital and community settings, jeopardizing empirical treatment of community-onset infections which is very alarming. (32). Also, SA002 had AMR gene family rifamycin-resistant beta-subunit of RNA polymerase (rpoB) whose resistance mechanism is antibiotic target alteration and antibiotic target replacement. It also had an H481N mutation which confers high-level rifampin resistance. Mupirocin is not commonly used in Kenya as compared to linezolid, thus detecting resistance genes to mupirocin is a great concern, considering it is from a community-onset. This new resistance being detected from community isolates is a public health threat and also poses a serious challenge for clinicians in treating CA-MRSA infections as was also described by Singh et al. (33). This further highlights a wider spread of undetected genetically encoded resistance, which is a concern since most health facilities do not perform genotypic tests for antimicrobials during routine patient management. This suggests that there could be more genetically encoded resistance that has not been detected and documented, which may affect the effectiveness of antimicrobials, thus affecting treatment outcomes.

Notably, mutations were detected within *gyrA* genes, and plasmid-mediated *dfrB* and *dfrG* genes. Reports have indicated that the *dfrG* gene is increasingly being documented in Africa, and is associated with travelers, though more research needs to be done (34).

Genomic analysis revealed that novel ST 7460 had SCC*mec* IV 2B, which is classically associated with CA-MRSA, matching sample source which is from community-onset wound infection (i.e., SSTI). The new ST 7635 isolate had SCC*mec* type III (3a) that is classically associated with HA-MRSA, also from community-onset wound infection (SSTI), which shows evidence of the correlation between CA-MRSRA and HA-MRSA, where either one can be isolated from a community setting or hospital setting (35). Virulence factors observed showed several genes important in *Staphylococcus* survival in its hosts such as inhibiting both innate and adaptive immune responses, enhancing *Staphylococcus* pathogenicity, and production of bacterial anti-inflammatory agents and chemotaxis inhibiting protein. These include (i) *sbi*, which inhibits both innate and adaptive immune responses, (ii) *spls* which induce and enhance *Staphylococcus* pathogenicity, and (iii) *scn* plays a role in *Staphylococcus* host immune evasion and is also a staphylococcal component inhibitor, (iv) *hlg* known to have membrane damaging factors, (v)

TABLE 3 AMR gene families detected in SA002 and SA004.

AMR gene family	Organism	Antibiotic class target	Resistance mechanism	Antibiotic resistance ontology and some of the functions	
ATP-binding cassette (ABC) antibiotic efflux pump	SA002 SA004	Fluoroquinolone antibiotics, Cephalosporins, Penam, Macrolides, Aminoglycoside, Oxazolidinone antibiotic, Diaminopyrimidine antibiotic, Phenicol antibiotic, and Tetracyclines	Antibiotic efflux	mgrA	Indirectly regulates norA (fluoroquinolone resistance gene) and tet38 (tetracycline resistance gene) expression. The gene also modulates clumping and virulence of *Staphylococcus aureus*
					Indirectly regulates norA (fluoroquinolone resistance gene) and tet38 (tetracycline resistance gene) expression. The gene also modulates clumping and virulence of *Staphylococcus aureus*
Major facilitator superfamily (MFS) antibiotic efflux pump			Antibiotic efflux	mgrA,	
				arlS,	Activates expression of mgrA and also regulates oxacillin resistance in MRSA
				norC,	Induces nucleosome sliding Is also a non-coding RNA that regulates the transcription of rRNA
				Staphylococcus aureus LmrS	Is a multidrug efflux pump for lincosamide resistance protein in *S aureus*
Multidrug and toxic compound extrusion (MATE) transporter	SA002 SA004	Glycyclines and Tetracyclines	Antibiotic efflux	mepR,	Represses the expression of *S aureus* multidrug efflux pump gene, mepA
				mepA	Is an *S aureus* multidrug efflux pump
Small multidrug resistance (SMR) antibiotic efflux pump	SA002 SA004	Disinfecting agents	Antibiotic efflux	sepA	Facilitates biofilm maturation and intercellular adhesion.
Trimethoprim-resistant dihydrofolate reductase dfr	SA002 SA004	Diaminopyrimidine antibiotic	Antibiotic target replacement	dfrG	Plays a role in folate metabolism
APH (2"), AAC (6')	SA002 SA004	Aminoglycoside antibiotics	Antibiotic inactivation	AAC(6')-le-APH(2")-la	Confers high-level gentamycin resistance and its presence jeopardizes the use of gentamycin and other aminoglycosides
Fosfomycin thiol transferase	SA002 SA004	Fosfomycin	Antibiotic inactivation	*Staphylococcus aureus* FosB,	Is a fosfomycin-inactivating enzyme that modifies an antibiotic into a compound that lacks bactericidal properties
Blaz beta-lactamase	SA002 SA004	Penam	Antibiotic inactivation	PC1 beta-lactamase (blaZ)	Enhances antibiotic resistance by catalyzing the hydrolysis of beta-lactams
Methicillin-resistant PBP2	SA002 SA004	Penam	Antibiotic target replacement	mecA	Gene responsible for methicillin resistance in *Staphylococci*

(Continued)

TABLE 3 (Continued)

AMR gene family	Organism	Antibiotic class target	Resistance mechanism	Antibiotic resistance ontology and some of the functions	
Erm 23S ribosomal RNA methyltransferase	SA002	Macrolide antibiotic, lancosamide antibiotic, streptogramin A antibiotic, streptogramin B antibiotic	Antibiotic target alteration	ErmA	Confer macrolide resistance in *S aureus*
Fluoroquinolone resistant parC and Fluoroquinolone resistant grrA	SA004 SA002	Fluoroquinolone antibiotic.	Antibiotic target alteration	sdrM	Is a drug transporter, responsible for increased resistance to antimicrobials such as norfloxacin
antibiotic-resistant isoleucyl-tRNA synthetase (ileS)	SA002	mupirocin	Antibiotic target alteration	Staphylococcus aureus mupA conferring resistance to mupirocin	Confers high level mupirocin resistance
Cfr 23S ribosomal RNA methyltransferase	SA002	Lincosamide antibiotic, oxazolidinone antibiotic, phenicol antibiotic, pleuromutilin antibiotic, streptogramin antibiotic	Antibiotic target alteration	cfrA	Encodes mutation 23SrRNA at A2503 by a methyltransferase
Rifamycin-resistant beta-subunit of RNA polymerase (rpoB)	SA002	Rifamycin antibiotics	Antibiotic target alteration Antibiotic target replacement	Staphylococcus aureus rpoB mutants conferring resistance to rifampicin	Confers high-level rifampin resistance

TABLE 4 Restriction enzymes detected in both SA002 and SA004.

Enzyme	Gene	Function		Recognition sequence
Type 1	*S.Sau8532II*	specificity subunit	block horizontal gene transfer between MRSA	AGGNNNNNGAT
	M.SauTCHI	methyltransferase		CCAYNNNNNNTGT
	S.Sau20231II	specificity subunit		CCAYNNNNNNTGT
Type IV	*SauUSI*	methyl-directed restriction enzyme	important barriers to the transfer of plasmid DNA from other bacteria	SCNGS

luK also known as bacterial invasins, attack natural killer cells, phagocytes, T-lymphocytes, and dendritic cells, (vi) *aur* which regulates biofilm growth cycle of *S. aureus*, (vii) *sak* known to activate plasminogen into plasmin which digests fibrin clots and activates latent matrix metalloproteinases leading to extreme proteolysis, (viii) *adsA* which evades host immune responses by modulating host pro-inflammatory responses, resulting in a prolonged infection, and (ix) *chp* a known bacterial anti-inflammatory agent and chemotaxis inhibiting protein.

Plasmids detected carried resistance genes coding for several antibiotics but most interestingly was plasmid rep15 in SA002 that had resistance genes to amphenicols, and linezolid (cfr gene) (expressed both phenotypically and genotypically) and mupirocin (mupA gene, expressed genotypically with no phenotypic break points in Vitek). Some of these antibiotics are not available in the country such as mupirocin, and this raises concern. Genetic characterization showed that the isolate had type I and type IV restriction enzymes that block gene transfer. The presence of a plasmid (rep15) containing resistance genes may suggest mutations affecting the functioning of these enzymes. Interestingly, AMR gene families; ATP-binding cassette (ABC) antibiotic efflux pump and major facilitator superfamily (MFS) antibiotic efflux pump that are multidrug efflux transporters and Cfr 23S ribosomal RNA methyl-transferase whose mode of action is antibiotic target alteration, could also play a role in linezolid resistance. Besides antibiotic efflux and 23S rRNA methylation mediated

by the *cfr* gene, linezolid resistance may arise from point mutations in the 23S rRNA proteins L3, L4, and L22 as well as point mutations in domain V of the 23S rRNA gene. However, no mutations in the 23S rRNA were detected and an alignment of corresponding amino acid sequences for L3, L4, and L22 between SA002 and the USA300 reference showed no amino acid mutations. Perhaps this is the first report of linezolid resistance from Kenya, though it is quite likely the resistance might be existing undetected so far. Also, AMR gene family antibiotic-resistant isoleucyl-tRNA synthetase (ileS) whose mode of action is antibiotic target alteration and carries antimicrobial resistance ontology *Staphylococcus aureus* mupA conferring high-level resistance to mupirocin could play a role in mupirocin resistance (**Table 3**).

IS256 was detected in SA002 plasmids rep7a and rep 15 in the reverse and forward strands. Its implication clinically results in increased hypervirulence and cytotoxin production, and also heightened pathogenicity. Also, it results in to change in the promoter sequence of the repressor of toxins which is a master transcriptional regulator responsible for the expression of virulence factors in *S. aureus* (36).

The new resistance observed and the novel STs reported in this study highlight evolution within *S. aureus* and *S. aureus* infections, even though the evolution is not a parallel emergence from MSSA lineages but evolution within the clonal complex. The tree topology is in support of the classification that places ST 7460 and ST 7635 in CC8. The clinical significance posed further highlights the need for alternatives to antibiotics as the rate of resistance in hospital and community settings is superseding the rate of manufacturing new antibiotics. This is a clear indication that interventions need to be put in place. Also, molecular epidemiological studies are needed to reveal unidentified STs and clonal types, with key emphasis on genomic mutations that will have public health significance (e.g., the detection of IS256 in SA002, similar to that of USA500, and the clinical impact it carries) and identify new resistance to higher drugs such as linezolid and, mupirocin. Also, surveillance studies focusing on mutations within resistance genes with clinical relevance, emphasizing community settings are needed. Community isolates observed in this study seem to be showing resistance that has not been reported before in the country (e.g., mupirocin) and resistance that has only been reported before in hospital settings (e.g., rifampin). Infection prevention and control measures that are done in hospital settings need to be done in the community settings with the same measures to effectively prevent antimicrobial resistance spread between the two settings. Antimicrobial stewardship should focus on research of a cost-effective molecular method to include genotypic AMR tests in routine patient management that will not affect turnaround time and will give results promptly for better patient management.

Conclusion

The appearance of linezolid resistance in SA002 adds to the increasing reports of linezolid resistance in different parts of the world. Due to excellent oral bioavailability, it is likely to be used for the management of outpatients with MRSA further creating pressure for the emergence of resistance. Linezolid is a reserved category antibiotic, much in need of preservation for serious clinical infections, and thus its resistance is important as the genotype observed was borne on a mobile element raising the possibility of rapid spread to other strains and/or species. Further, linezolid resistance could arise in settings of limited clinical use and should therefore be investigated from a One Health perspective. Delineation of HA-MRSA and CA-MRSA is becoming clinically challenging and molecular characterization helps to understand better the transmission dynamics of the pathogen. Data obtained provided preliminary insights into unidentified STs of CA-MRSA in Kenya. More studies in Africa need to be community-focused to reveal circulating strains and antimicrobial resistance that may have spread between hospital and community settings. Establishing AMR programs that enhance data sharing and information use, aimed at keeping track of new emerging resistance in hospital and community settings in Africa, need to be done. Novel strains detected have shown resistance to broad-spectrum anti-staphylococcal antibiotics not commonly used in the country, thus jeopardizing the successful treatment of MRSA infections. Given that most health facilities do not perform genotypic susceptibility tests for a routine patient management, genotypic resistance may go unnoticed in the continued absence of such programs.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving human participants were reviewed and approved by Aga Khan University, Nairobi. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

GR and JNj: conceptualization. JNj and AM: methodology. GR, CN, and GO: supervision. JNj: writing –

original draft. JNj, JNy, ZM, and GR: writing review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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The role of telepathology in improving cancer diagnostic and research capacity in sub-Saharan Africa

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Non-communicable disease (NCD), including cancer, disproportionately affect Low- and Middle-Income Countries (LMICs). This inequity is in part due to limitations of pathology services, both human and infrastructural. While significant improvements have been made to address these gaps, creative approaches that are mindful of regional priorities, cultural differences, and unique local challenges are needed. In this perspective, we will describe the implementation of telepathology services in sub-Saharan Africa (SSA) that serve as cornerstones for direct patient care, multi-disciplinary care coordination, research programs, and building human capacity through training. Models and challenges of system implementation, sustainability, and pathologist engagement will be discussed. Using disease and site-specific examples, we will suggest metrics for quality control and improvement initiatives that are critical for providing high-quality cancer registry data and necessary for future implementation of therapeutic and interventional clinical trials.

KEYWORDS

telepathology, cancer, low- to middle-income countries (LMICs), pathology, Africa

Introduction

The impact of non-communicable diseases (NCDs), particularly cancer, represents a major health crisis in low- to middle-income countries (LMICs) that constitute the entirety of sub-Saharan Africa (SSA). In 2020, ∼60% of new cancer diagnoses occurred in LMICs (1), where only 5% of global spending is directed toward cancer control (2). Moreover, cancer-related deaths outnumber those related to HIV/AIDS, Tuberculosis, and Malaria (3) and by 2030, 75% of global cancer deaths will occur in LMICs (4). Social and financial consequences of the disproportionate premature deaths in LMICs pose a significant obstacle to social and economic growth and equity. While the reasons for these disparities are multifactorial, lack of timely and accurate tissue diagnosis represents a critical defect in effective systems of cancer care. Tissue diagnosis is necessary for the provision of cancer services to the individual patient but is also critical for the development of comprehensive national/regional cancer registries and clinical trial implementation, that in turn, inform and guide public policy.

These far-reaching and population-level impacts of effective pathology services include development of cancer registries, disease surveillance programs, and national preventative care strategies. In this perspective, we focus on the gaps in pathology services in SSA, progress in diagnostics largely driven by responses to communicable disease burden, and the development of pathology and telepathology programs that aim to improve cancer care in the region.

Human and infrastructural limitations to effective pathology services in SSA

Recent data from the Lancet Commission on global diagnostics has found that only 47% of the global population has access to laboratory medicine services (5). The critical need for pathology services and gaps to effective deployment of pathologists and equipment in SSA have been extensively described over the past decade (6–14). Survey data published in 2012 (11, 14) demonstrated that with the exceptions of Botswana and South Africa, all countries in SSA have fewer than one pathologist for every 500,000 persons. Many countries have one per million people, and Somalia at that time had no pathologists. For reference, in 2010 the US had an estimated one clinical or anatomical pathologist for approximately every 20,000 persons. The lack of laboratory facilities, reagents and other supplies, advanced technology, infrastructure, supportive staff, and trained laboratory technicians further widens the gap (15). The education of physicians stalls behind more developed countries, leaving a doctor shortage that is not easily compensated. One estimate anticipates that it would take over 400 years to train the number of pathologists needed to have an adequate diagnostic workforce in the current educational system (11).

With respect to human capacity, the divide between the relative resource-rich and resource-poor countries within the region is not necessarily improved by current training paradigms. Physicians who must leave their country of origin to complete pathology programs in high-resourced settings or in high income countries (HICs), find themselves in a challenging situation after training. With newly gained skill and developing expertise in state-of-the art diagnostics, returning to their home country to practice under significant limitations in pathology capacity beyond basic histology and without access to ongoing educational activities for growth and development is challenging. This "brain-drain" has been documented for physicians leaving SSA as a whole (16–19), but also occurs *within* the region with many health workers moving from the public to the private sector, or from rural to more developed urban areas. Furthermore, those who do return are often promoted to leadership and administrative positions that limit time and effort to devote to the clinical practice of pathology or to developing cancer research programs. In addition, they are more likely to suffer from increased stress and burnout (20).

Infrastructural limitations inhibiting ability to provide timely and accurate diagnoses consists of many challenges including lack of physical laboratories with sufficient equipment, reagents, and capacity to process patient samples, among other things (21). Availability of diagnostic tests varies between LMICs and is relative to how many primary care vs. advanced care facilities are available (13). Major gaps in availability are most pronounced in the local primary care settings.

To help address these shortages, the WHO created a list of priority medical devices for cancer management in 2017 (3) with the goal of increasing access especially in LMICs. Pathology and laboratory medicine services are covered under a separate section and consists of an exhaustive list of instruments, reagents, and personnel supply that are the basic minimum needs for providing cancer care. When broken down to testing needs by tier of laboratory, the costs range from modest to enormous from the primary setting to the national/regional referral center respectively (21). Coupled with the essential treatment lists, a clear plan to cover basic need has been created (22). However, a lack of population disease data and continued underinvestment of pathology services in LMICs makes exact quantification of costs to meet the need difficult.

Interventions and the importance of leap frogging

Interventions must be multifaceted and collaborative to face this complex challenge. The answer is not going to be as simple as training more pathologists in the current educational system in LMICs for instance. Insufficient educational infrastructure currently exists to train an adequate number of pathologists and highly skilled laboratorians using the current systems in place; so it is obvious that advanced education and access to trained pathologists outside of LMICs must occur concurrently for delivering equitable patient care to meet the immediate and future demand.

"Leap Frogging" is a concept that is often applied to global health and essentially means to find a solution to a problem that avoids previously unavoidable steps. A common example in pathology is the use of point of care (POC) testing, such as the Cepheid's Xpert MTB-RIF test that obviates the need for traditional reagents, instruments, laboratory facilities, highly trained technologists and so forth to deliver a rapid diagnosis of drug resistant tuberculosis (8). The medical community must continue to focus on technological advances that leap frog traditional methods of providing care and look for solutions that avoid historical obstacles altogether.

Role of telepathology

The role of *telepathology*, broadly defined as electronic "sharing" of histopathologic images and clinical data, has evolved in parallel with improvements in technology and communication, and expanded with lower production costs, philanthropic efforts, large-scale public-private partnerships that focus on closing the gap in low-resource settings, and collaborative research programs. Four types of telepathology platforms have been defined and include: (1) static imaging, (2) whole-slide scanning, (3) dynamic nonrobotic telemicroscopy, and (4) dynamic robotic telemicroscopy, and their selection is dependent on local need and/or available resources (23, 24). Unsurprisingly, considerations for platform selection and implementation include technical and resource constrains, including consistent power supply, reliable internet, access to supporting hardware (i.e., computers and servers), and trained staff. However, each of these formats has been used effectively in SSA, and some of these programs have been well-described in published literature (25–50).

Ultimately, deployment of telepathology for cancer care in SSA depends on long-distance engagement of trained pathologists and a sufficient source of funding for implementation. These programs exist on the spectrum from those primarily supported by organizations with international collaboration priorities funded by member and sponsor support to structured research programs funded through grants. Although it has been an excellent example of leap frogging access to care, it is by no means the catholicon solution. In brief, slides still have to be made which requires infrastructural and human capacity as discussed above. As they are rarely revenue-generating, sustainability of telepathology-based intervention programs relies on local, regional, and national governmental, as well as continued international support. A summary of the challenges and barriers facing pathologists in LMICs as well as the benefits and challenges of deploying telepathology in these settings is highlighted in Figure 1. Irrespective of their path to development and deployment, telepathology-based initiatives have significantly changed the face of clinical cancer care, pathology training, and cancer research in SSA. Below, we describe how use of telepathology has paved the way for advances in cancer care, improving pathology training, and expanding research access equity.

Clinical cancer care and pathology training

With the revolution of digital pathology, numerous telepathology solutions (51) for providing patient care have evolved to include solutions for global pathology delivery. Some of these projects have been initiatives started by large pathology organizations such as the ASCP/DUKE/UCSF/MOTIC (52) partnership that has successfully installed MOTIC slide scanners in multiple laboratories throughout Africa. The pathologists on site are then able to consult with volunteer pathologists around the United States for second opinions and/or aid with diagnoses. This has also served as a method of supplemental education for the pathologists in low resource settings. This is a major step forward since the pathologists working in LMICs usually lack access to continuing medical education in their daily practice. Another organization that has linked physicians working in LMICs with expert volunteers around the world is Project Echo (53).

Project Echo uses digital pathology and static images to hold multidisciplinary tumor boards that incorporate trainees and mentoring opportunities as a way to educate and support colleagues in low resource settings. They now have 146 programs in 13 different countries and have reported amazing success.

Other groups and individuals have harnessed the power of the internet to deliver high quality didactics aimed at pathologists who have limited access to advanced training opportunities and continuing medical education. Dr. Jerad Garner is a US based soft tissue and dermatopathologist who creates educational content on YouTube and reports frequent views from pathologists in LMICs. For years, he has also led and participated in Facebook discussion groups (Facebook, Inc., Menlo Park, California) to educate patients with rare diagnoses.

Dr. Kamaljeet Singh is a US based breast pathologist that has worked with learners in various sites across the US and internationally to facilitate case-based learning while in-person opportunities were stunted by the COVID pandemic. He and his team used Google Classroom (Google, LLC, Mountain View, California) technology to accomplish this and reported positive feedback from the learners in the program (54).

In response to stifled educational opportunities for medical students to gain practical access to pathology rotations during the COVID pandemic, Dr. Kamran Mirza and Cullen Lilley created a virtual medical student shadowing experience. Participants can create a free account on www.PathElective.com and virtually participate in pathologist-led courses that were curated specifically for medical students, and they even have the opportunity to earn certificates of completion.

A newly formed group of pathologists from around the world have collaborated online to form a new organization, called Open Pathology Education Network (OPEN) aimed at educating pathology residents in low-resource settings. The group will focus its efforts on supporting colleagues in LMICs by providing access to free high-level training using digital pathology, the internet, and video-conferencing among other virtual tools.

It is worth noting that prior to the internet, there were pathologists working to support colleagues in low-resource settings such as Dr. David Kaminsky who started "Africa Calls," a telephone-based case consultation service. These are just a few examples of some of the ways that organizations and pathologists around the world have collaborated to improve patient care.

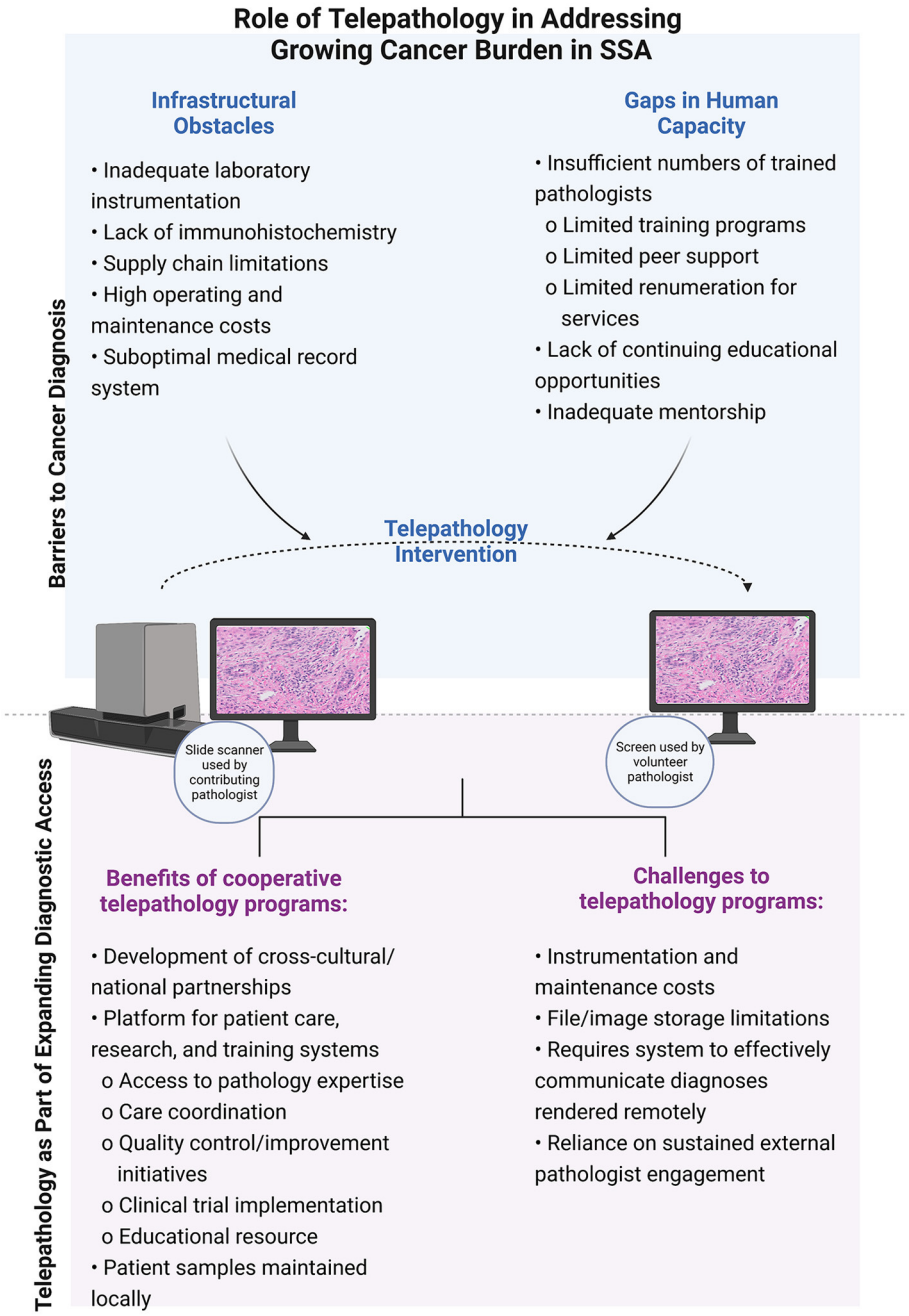

FIGURE 1
The role of telepathology in addressing the growing cancer burden in sub-Saharan Africa.

Other methods of improving patient access to care are on the rise, such as the advances in Artificial Intelligence (AI) as applied to image based diagnostic fields of medicine such as radiology and pathology. These innovations have begun to be incorporated into global health care strategic planning. For instance, USAID has identified AI as a tool to leap-frog traditional diagnostics, bypassing the need for microscopy as the primary screening mechanism for each slide. Instead, they have proposed that AI screens of digitized slides to identify areas of interest for clinician review, or even infer diagnoses directly using machine learning and pattern recognition (55).

One gap that remains in SSA is limited pathology laboratory participation in External Quality Assurance (EQA) programs for whole slide imaging of histology and cytopathology samples. EQA programs in Africa for epidemic-prone diseases (enteric diseases, meningitis, plague, tuberculosis, and malaria) and

blood samples are robust but oncologic EQA lags behind that in HIC (56). EQA providers, such as the College of American Pathologist have put forth validation guidelines for whole slide imaging systems for diagnostic purposes (57). However, the feasibility of implementing such extensive EQA schemes in SSA for cancer care, where laboratories can be tested against their peers is currently limited as they are associated with high costs and require broad collaboration throughout the region. Proficieny testing programs, where external referees send test samples for identification and provide feedback, would be an appropriate benchmark measurement for SSA pathologists that would help identify gaps in cancer training and likely improve diagnostic accuracy.

Research and research capacity development

Cancer research output and cancer control programs developed from HICs are not uniformly globally generalizable. Given the unique context, including differences in environmental exposures, oncogenic pathogens, and social, political, and economic pressures, distribution of cancer types, and, indeed cancer biology, may be distinct. Collaborative cancer research programs mindful of local priorities and needs in (and *within*) SSA are critically important for the development of robust cancer registries (58), disease surveillance programs and national preventative care strategies. To this end, telepathology can serve as a centerpiece for cancer research programs in LMICs and as a platform for research mentorship.

Research programs in LMICs have the unique ability to improve health outcomes while also empowering the next generation of local researchers and strengthening research capacity (59). The University of North Carolina Project Malawi Cancer Program uses telepathology to collaborate between physicians and researchers in Malawi and the United States (29). The program was originally developed to accurately diagnose and classify hematologic cancers for patient enrollment into an ongoing observational Kamuzu Central Hospital (KCH) Lymphoma Study, as reported previously. In addition to care coordination and patient follow-up, the weekly telepathology-based meetings, modeled after multi-disciplinary tumor boards, serve to ensure diagnostic accuracy and identify cases and case series for further investigation (50).

Over the course of the KCH Lymphoma Study, initiated in 2013, the format of telepathology research conferences has evolved with changes in staffing and infrastructure. Originally, glass slides scanned in Malawi were presented by US-based pathologists through a Virtual Private Network connection to support primary diagnosis, and aid in interpretation of newly deployed immunohistochemistry. With additional research funding and expansion of diagnostic services, additional Malawian pathologists with advanced training and expertise were employed through government and university support. Telepathology conference "leadership" was shifted to Malawian pathologists who now are the primary drivers of the scanned images shared *via* a readily available video platform. US-based pathologists serve as consultants and second reviewers for study enrollment purposes (50). Of critical importance, is that tissue blocks of enrolled patients are shipped to UNC on a quarterly basis for quality control (QC) and improvement (QI) initiatives and further studies not currently available in Malawi. This form of central review has identified a high concordance rate as well as identified gaps that have led to improvements in Malawi laboratory workflow. Such QC/QI initiatives are important for quality research programs, clinical care, and laboratory accreditation.

In addition to the primary aims of the KCH Lymphoma Study which rely on accurate classification, observations of clinically unique cases or case series have emerged from the telepathology program. These have, in turn, served as primary output for Malawian early-career cancer investigators who aim to develop independent research careers and successfully compete for independent funding. The pathologists themselves are now heavily involved in programmatic development, research output, and local cancer registry leadership.

Other telepathology programs in other LMICs, have developed models based around collaborative telepathology systems. A recent successful example is the collaboration between The City Cancer Challenge Foundation (C/Can) and the American Society for Clinical Pathology in their work establishing a telepathology platform in Yangon, Myanmar (60). The program was based off an extensive needs-assessment at the local level and development of a site-specific intervention with an in-depth analysis of intervention outcome that followed. It serves as an excellent model of project development and deployment.

As noted above, there are challenges and obstacles to research-based telepathology programs. While the questions being addressed are mindful of local need, programs rely on grant funding and investigator time/availability. Often, the time from grant application to grant funding and necessary regulatory approval is prolonged. Moreover, sustainability and growth requires sufficient research output and broadening of programmatic goals for additional grant application, and diversification of funding sources (29), including local governmental support and engagement.

Telepathology can be a critical service when establishing research programs in an LMIC. It provides the infrastructure from which improved training, data collection, and research capacity can take place, all while ensuring local ownership of the research (61).

Conclusions

There is inadequate access to tissue-based diagnostics in Africa. To ameliorate the dire need for interventions, the

solutions to delivering equitable healthcare must be equally as multidimensional and will necessitate a unified collaborative approach to ensure success. Although telepathology has ushered in a groundbreaking means of improving access, collaborating with global colleagues and developing research programs, it cannot comprehensively address dire gaps in caner care delivery. Innovative and collaborative solutions and continued advocacy for global diagnostics remain critically important.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

DR, KP, TT, and YF were responsible for developing, writing, and reviewing the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Clinicopathologic features of renal cell carcinomas seen at the Aga Khan University Hospital in Kenya

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Introduction: Kidney cancer accounted for 1. 8% of global cancer deaths according to Globocan 2020 estimates, with most of these being renal cell carcinomas. Lower rates of renal cell carcinoma are reported for Africa and these are expected to change for a combination of reasons. The clinical and morphologic characteristics of renal cell carcinoma seen within Kenya have not been described before. This study aims to partially fill this gap.

Materials and methods: This was a cross-sectional descriptive study examining electronic histopathology reports from the Aga Khan University Hospital Nairobi Laboratory for the period January 2016 to May 2022.

Results: Sixty cases of renal cell carcinoma were identified. The mean age at diagnosis was 55.3 years. The most common histologic subtype diagnosed was clear cell renal cell carcinoma (41.7%), followed by papillary renal cell carcinoma and renal cell carcinoma not further specified (both 21.7%), and chromophobe renal cell carcinoma (11.7%). The most frequent specimen type was resection, followed by cores of renal masses. The mean tumor size was 8.5 cm. Sixty-seven percent of patients presented with Stage III and above.

Discussion: Renal masses were the commonest clinical indication for biopsy among the records reviewed. The male to female ratio, as well as the mean age at presentation were comparable to what is described in literature for other regions of the world. The proportions of the commonest histologic subtypes matched what is described in other parts of the world. Challenges in the identification of histologic subtypes included having a limited panel of antibodies for diagnosis and the lack of genetic molecular tests for histotyping.

Conclusion: The spectrum of histologic subtypes of renal cell carcinoma seen at a tertiary referral hospital in Nairobi, Kenya was similar to that described in other parts of Africa and the globe. The age at presentation with renal cell carcinoma was consistent with what has been described in literature. Challenges were identified in the accurate histotyping of renal cell carcinoma due to constrained resources. Majority of cases diagnosed presented at advanced stage.

KEYWORDS

renal cell carcinoma, oncopathology, Kenya, genitourinary pathology, clear cell renal cell carcinoma

Introduction

Kidney cancer is the 14th most common cancer by incidence and accounted for 1.8% of global deaths from cancer according to Globocan 2020 estimates (1), with most of these cancers being renal cell carcinomas (RCC). Globocan 2020 estimates that kidney cancer represented 1.1% of new cancers and 1.1% of deaths from cancer in Kenya (2). Most early-stage RCC are discovered on routine imaging for other diseases or patient complaints. Less than 20% of patients present with pressure (flank pain, abdominal fullness or swelling), urinary tract symptoms (bleeding, repeated infections from obstruction) or paraneoplastic syndromes (3). Of note, up to 20% of patients may present initially with metastatic disease to various sites (4, 5).

Risk factors for RCC include advancing age, male sex, smoking, and obesity. The global age standardized incidence rate for renal cancer per 100,000 is 6.1 for males and 3.2 for females (1). Dietary risk factors such as vegetable or meat intake have shown conflicting relationship with incidence of RCC in various studies (3). Acquired cystic kidney disease in patients on dialysis is also an established risk factor (6). Less commonly, rare genetic syndromes are associated with RCC including von Hippel-Lindau syndrome, Hereditary papillary renal carcinoma, Birt-Hogg-Dube syndrome, Succinate dehydrogenase (SDH)–deficiency, Hereditary leiomyomatosis and renal cell cancer and Hereditary renal carcinoma (HRC).

The highest incidence of renal cell carcinoma has been observed in Belarus and North America with lower rates reported among populations in Africa (3, 7). These reportedly lower rates are likely a reflection of disparities in access and provision of allopathic healthcare. One of the consequences of this is under-reporting because of inadequate infrastructure that leads to underperformance of cancer registries (4). Thus, this low incidence of RCC in Africa may represent an underestimation. In multiracial populations, it has been observed than individuals with African ancestry have an increased risk for RCC and worse outcomes (7). The World Health Organization (WHO) estimates that 1 in 5 adults and 1 in 10 children and teenagers will be obese by December 2023 in 10 high-burden African countries (8). There is already an established link between being overweight and obese and the risk for developing renal cell carcinoma. It is therefore expected that this increase in the number of obese and overweight individuals will lead to an increase in the incidence of RCC. It is also possible that the incidence of RCC will increase in sub-Saharan Africa, in the coming years, with increasing use of radiologic imaging for various other conditions as this becomes more available.

There are several distinct histologic subtypes of RCC that have been described, with distinct clinical behavior including presentation, aggressiveness, and response to targeted therapy (9). In addition, the current WHO 2022 classification of urinary and male genital tumors includes entities that are defined by molecular genetic abnormalities (9). This makes it challenging to accurately diagnose these tumor entities because the necessary molecular tests are not widely available, particularly so in low- and medium-income countries (LMICs).

The most common subtypes of RCC described include clear cell (75–85% of all RCC), papillary (10–15%) and chromophobe (5–10%). Of these, clear cell renal cell carcinoma tends to have a worse prognosis than most of the other subtypes, and a more aggressive clinical course. The other subtypes with bad prognosis are equivalent to the prognosis of clear cell renal cell carcinoma. Some of the rare histologic subtypes such as clear cell papillary RCC, have indolent behavior and this is now categorized as a tumor rather than carcinoma (9).

Management of renal cell carcinoma is dependent on the histologic subtype and the stage at disease presentation, with only surgery being curative for early and localized disease. There are several approved targeted therapies that may also be used in the adjuvant setting subsequently. Treatment of advanced renal cell carcinoma is supportive, although many new targeted treatments are under investigation (10, 11). Access to these targeted therapies remains a challenge in LMICs due to the prohibitive cost.

The Aga Khan University Hospital Nairobi Main Laboratory is part of a private nonprofit institution, the Aga Khan University Hospital. The anatomic pathology laboratory processes 14–16,000 surgical specimens annually, covering all areas of surgical pathology practice. The laboratory receives and reports specimens from the parent hospital as well as several private, faith-based and government institutions.

There is an epidemiological need to define the clinical and morphologic characteristics of renal cell carcinoma seen within Kenya specifically, and the East Africa region in general, as these data do not exist to the best of our knowledge. This clinicopathologic review aims to partially fill this gap. It is hoped that this information will inform policy and guide the allocation of resources such as training in oncology care, and the development and refinement of treatment strategies.

We aimed to describe the clinical and pathologic characteristics of renal cell carcinoma as seen at Aga Khan University Hospital (AKUHN) from January 2016 to May 2022. This description includes the presentation and diagnostic work up where available and/or relevant.

TABLE 1 Age at diagnosis of renal cell carcinoma.

	Number of patients	Youngest	Oldest	Mean	Std. Deviation
Males	33	21	85	60.7	16.9
Females	27	26	86	48.7	18.4
Combined	60	21	86	55.3	18.5

Materials and methods

This was a cross-sectional descriptive study examining electronic histopathology reports from the Aga Khan University Hospital Nairobi Main Laboratory for the study duration. All renal cell carcinomas diagnosed in that period were included in the study. Data collected was entered in Microsoft Excel 2021 sheets. Data analysis was performed using Excel and SPSS version 20. The most relevant study limitation was finding incomplete reports.

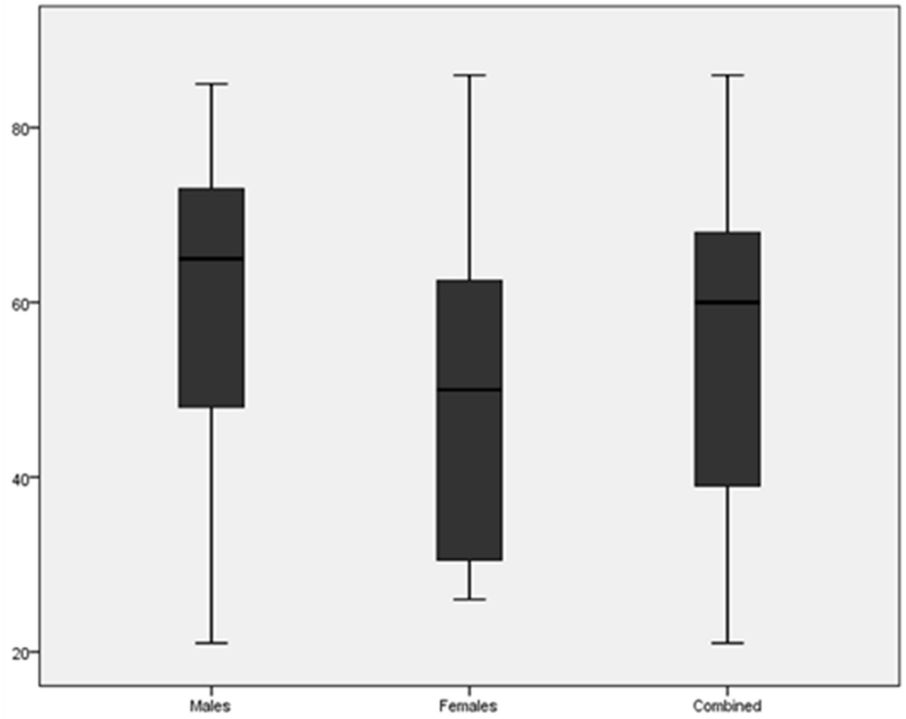

FIGURE 1
Boxplot comparing the age at diagnosis of renal cell carcinoma for males, females and both combined.

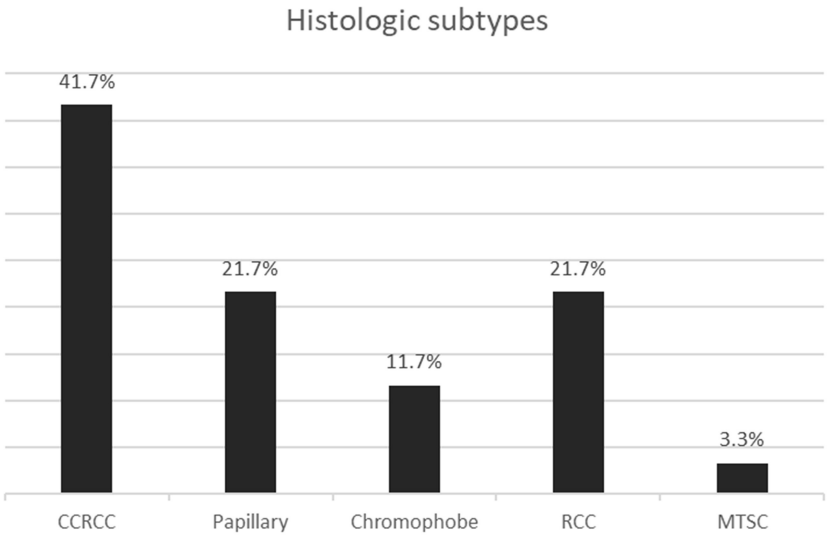

FIGURE 2
Bar graph showing the histologic subtypes of renal cell carcinoma. CCRCC, Clear cell; RCC, Renal cell carcinoma not otherwise specified; MTSC, Mucinous tubular and spindle cell.

Results

Records were available for 60 patients with a histopathologic diagnosis of renal cell carcinoma, six of which were of biopsies from metastatic sites.

The clinical presentation was recorded for 45 cases. The commonest presentation of patients with RCC was a renal mass in 29 patients (64%, 95% CI 49–78), followed by flank pain in 8 patients (18%, 95% CI 8–32) and hematuria in 3 patients (6%, 95% CI 2–18). Many patients had more than one presentation recorded e.g., renal mass and hematuria and others had a single unique presentation such as metastasis to a unique site.

RCC was diagnosed with slightly higher frequency in males than females with a male to female ratio of 1.22:1.

The average and median age at diagnosis of RCC are shown in Table 1 and compared in Figure 1. The mean age at presentation with renal cell carcinoma was older for males by 12 years compared to females.

The most common histologic subtype diagnosed was clear cell RCC. The next most frequent were papillary RCC and RCC not otherwise specified, as shown in Figure 2. Only two of the papillary RCCs were type 1, the rest being either type 2 or mixed type 1 and 2.

Tumor size was available for 32 resection specimens as shown in Table 2.

The status of the margins was available for 29 of the resection specimens. Most (22 cases) had achieved clear margins. The positive margins were Gerota fascial margin (4 cases), renal vein (3 cases), renal sinus, perinephric fat, and parenchymal margins (1 of each).

Data on specimen type, tumor laterality, histologic grade, and stage of carcinomas are shown in Table 3. Only 3 cases had lymph nodes removed for staging.

Twenty-seven of the cases had immunohistochemistry done either for histotyping or to identify the primary in cases of metastatic disease, based on clinical and imaging suspicion. The characteristics of these tumors are shown in Table 4.

Three of the metastatic sites biopsied were liver, making this the commonest site of metastasis that had been biopsied. Other metastatic sites included a scapular mass, lung, and a mesenteric nodule.

TABLE 2 Tumor size for resection specimens ($N = 32$).

	Smallest	Largest	Mean	Median	95% CI
Tumor size (cm)	3.5	18	9.7	8.5	1.4

TABLE 3 Data on specimen type, tumor laterality, histologic grade, and tumor stage.

Variable	Value	Number (%, 95%CI)
Specimen type ($N = 60$)	Nephrectomy (Simple or unspecified)	21 (35%, 23–48)
	Radical nephrectomy	13 (21.7%, 12–34)
	Renal core	16 (26.7%, 16–40)
	Partial nephrectomy	2 (3.3%, 0.4–12)
	Other core	5 (8.3%, 2.8–18)
	Other	3 (5%, 1–14)
Laterality ($N = 49$)	Right	25
	Left	24
Histologic grade ($N = 39$)	1	10 (26%, 13–42)
	2	18 (46%, 30–63)
	3	5 (13%, 4–27)
	4	6 (15%, 5–30)
Stage (AJCC prognostic groups) ($N = 46$)	I	9 (20%, 9–34)
	II	6 (13%, 5–26)
	III	14 (30%, 18–46)
	IV (Locally advanced)	6 (13%, 5–26)
	IV (Metastatic)	11 (24%, 13–39)

Discussion

A renal mass was the most common clinical presentation recorded in the reports, it is possible that the renal mass was found following investigation for the symptoms of abdominal fullness, pain, or hematuria. This sequence of preoperative clinical investigation was not necessarily captured in the request form accompanying the specimens submitted for evaluation.

The ratio of males to females in our study population was 1.22:1, which is compares to the described global ratio of 1.58:1, and therefore consistent with what is known that renal cell carcinoma has a slight male preponderance (1).

The mean age at which renal cell carcinoma was diagnosed was 55.3 years which is similar to what has been described in literature. More than 77% of kidney cancer patients are aged older than 50 years (12). The age also varied between men and women with males presenting at a slightly older age group, again similar to what has been described for global data (12).

The commonest histologic subtypes seen were similar in proportion to what has been described in other parts of the world and Africa in particular. It is noted that the current (2022) WHO Classification does not recommend grouping of papillary RCC into types 1 or 2, and the data presented represent the prior historical classification. Table 5 shows data from other studies in various parts of the African continent.

There were challenges in the diagnosis of RCC that led to a proportion of tumors being categorized as RCC, not otherwise specified. Although the available panel of antibodies for immunohistochemistry was usually adequate to make a diagnosis of renal cell carcinoma, it was sometimes

TABLE 4 Clinicopathologic characteristics and immunoprofile of renal cell carcinomas.

Case	Diagnosis	Age	Sex	Specimen	Pathologic Stage	Positive IHC stains	Negative IHC stains
1	RCC	39	F	Scapular mass	M	AE1/AE3, Vimentin, EMA, E-cadherin	TTF1, HER2
2	Chromophobe	31	F	Nephrectomy	T2bNX	CK7	CD10, Vimentin, CD117, RCC, Inhibin
3	CCRCC	63	M	Mesenteric nodule	M	AE1/AE3, Vimentin, CD10	EMA, Desmin, S100, CD117
4	CCRCC	79	M	Renal core	CD10, Vimentin		
5	Chromophobe	27	F	Radical nephrectomy	T1bNX	CK7, RCC	Vimentin, CD10
6	CCRCC	63	F	Renal core	CD10, Vimentin		
7	RCC	60	M	Lung core	M	CD10	TTF1, CK7
8	RCC	60	F	Renal core	Vimentin	CK7	
9	RCC	74	M	Renal core	CK7	Synaptophysin, RCC, CD10, Inhibin	
10	Chromophobe	30	M	Radical nephrectomy	T1	CK7	Vimentin
11	Chromophobe	77	M	Renal core	CK7, CD117	Vimentin, CD10	
12	Papillary	37	M	Renal core	CK7, AMACR, CD10		
13	CCRCC	30	M	Nephrectomy	T1bNX	Vimentin	CK7, CD10, RCC
14	Papillary	45	M	Nephrectomy	T1bNX	CD10, AMACR, CK7	
15	RCC	66	M	Renal core	CK7, EMA, Vimentin, CD10	CK20	
16	MTSC	64	F	Nephrectomy	T2bNX	AE1/AE3, AMACR, CK7	CD10
17	Papillary	27	F	Renal core	M	AE1/AE3, AMACR, Vimentin	CK7, CD10
18	RCC	54	F	Liver core	M	CK7	CK20, Synaptophysin, HepPar1
19	RCC	85	M	Liver core	M	CK7, CD10,	HepPar1, p63
20	CCRCC	26	F	Nephrectomy	T3aNX	AE1/AE3, CK7, CD10, Vimentin	WT1, S100
21	RCC	42	M	Renal core	M	CD10, Vimentin, AMACR	CK7, CD117,
22	Chromophobe	65	F	Renal core	CK7, CD10	Vimentin	
23	CCRCC	54	F	Nephrectomy	T1a	AMACR, Vimentin, CD10, weak AE1/AE3	CK7
24	Papillary	79	M	Renal core	AE1/AE3, CK7, Vimentin, AMACR	S100	
25	Chromophobe	84	M	Radical nephrectomy	T3aN0	CK7, CD117	Vimentin
26	MTSC	48	M	Radical nephrectomy	T3aNX	CK7, AMACR	
27	Papillary	61	M	Renal core	CK7, Vimentin	S100, CD117	

CCRCC, Clear cell renal cell carcinoma; RCC, Renal cell carcinoma, not otherwise specified; MTSC, Mucinous tubular and spindle cell RCC.

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TABLE 5 Renal cell carcinoma clinicopathologic features in studies from Africa.

References	Study type	Year	Location	Number of cases	Age (years)	Commonest RCC (%)	Pathologic Stage ≥3
Amenu et al. (13)	Retrospective	2020	Ethiopia	64	Median 53.4	CCRCC 55, ChRCC 20, PRCC 18	34%
Atanda et al. (14)	Systematic review	2017	Nigeria	443	Mean 45 ± 4	CCRCC 60–85.7, PRCC 23.8–46.2	80–96%
Du Plessis et. al (15)	Prospective observational	2020	South Africa	31	Mean 56.39 (SD 10.16)	CCRCC 74, PRCC T2 16, PRCC Mixed 3, ChRCC 7	
Salako et al. (16)	Retrospective	2017	SW Nigeria	51	Median 41.7	CCRCC 60.8, ChRCC 17.6 PRCC13.7, Sarcomatoid 3.9, Mixed 1.9	52.9%

CCRCC, Clear cell RCC; PRCC, Papillary RCC; ChRCC, Chromophobe RCC.

limited for histologic subtyping. The following antibodies were not available: carbonic anhydrase IX, TFE3, SDHB and TFEB. It should be noted that the latter three are used to diagnose very rare histologic subtypes. On the balance however, this may not always influence patient care adversely given that surgical resection is the mainstay of management for localized disease regardless of the histologic subtype.

Additionally, the current WHO Classification has a category of molecularly-defined RCC which cannot be diagnosed as such without the use of ancillary molecular genetic testing. Although in many instances there are morphologic clues to suggest some of these entities (papillary morphology in a young person and weak staining for keratin suggesting MiT family translocation RCC, flocculent cytoplasm suggesting SDH-deficient RCC), ultimately the definitive diagnosis requires molecular genetic testing and the availability of this remains a challenge. This becomes an area of potential future collaboration and capacity building, both in the performance and interpretation of molecular genetic testing. This capacity would be useful in molecular oncopathology of tumors from other sites as well, for diagnosis, prognosis and even therapy.

The other challenge seen with histologic typing is the changes in tumor morphology that would occur due to poor fixation of a large tumor mass. Several of the nephrectomy specimens were obtained from referring faith-based and government health facilities which were not nearby. This meant that some of the specimens had to be transported for several days before they were processed. If the entire nephrectomy specimen is placed in formalin without prior dissection, fixation may not be optimal and partial autolysis in an already large vascular-compromised mass led to challenges in morphologic assessment as well as immunohistochemical staining.

The surgical treatment was adequate in many cases, with most of the resections having clear margins. The positive margins were noted, as expected, in patients with advanced stage disease, and probably represent the challenge of adequate resection with large tumor burden. Surgical resection is curative treatment for early and localized disease in many instances and this probably contributes to the overall low mortality of renal cell carcinoma in that clinical context.

Thirty-four (57%) of the specimens on which a diagnosis of renal cell carcinoma was made were complete resections of the involved kidney, with only two of the specimens in our series being partial nephrectomies. If as expected there is a future increase in the diagnosis of smaller, earlier stage tumors, then we may expect to see more partial nephrectomy specimens. The other reason why there might be a possible change to more partial nephrectomies is the fact that several of the risk factors for RCC (chronic kidney disease, hypertension and obesity among others) also compromise kidney function (17). This means that there is greater need to perform nephron-sparing surgery as much as possible which is also the approach recommended in some current management guidelines (10).

The next most common specimen received was core biopsies of renal masses, both from patients with disease limited to the kidney as well as some patients with metastatic disease in order to confirm the primary. This specimen type would also be expected to increase in number, given the increasing role of renal mass biopsy in the diagnosis of renal cell carcinoma. Renal mass biopsy is increasingly being used to make decisions regarding whether to pursue active surveillance or proceed with surgery for localized renal masses suspicious for carcinoma (18).

Sixty-seven percent of the cases of RCC in our series were diagnosed at advanced stage (Stage III and above). Although this was similar to other data reported on the African continent in Table 5, this proportion was much higher than that reported in Euroamerica, for comparison, where only 25% of patients present with advanced disease (19).

Conclusion

The spectrum of histologic subtypes of renal cell carcinoma seen at a tertial referral hospital in Nairobi

Kenya matched that described in other parts of Africa and the globe. The age at presentation with renal cell carcinoma was comparable to what has been described in literature. A challenge was identified in the accurate histotyping of renal cell carcinoma caused by lack of access to molecular genetic testing. It is expected that there will be an increase in the number of partial nephrectomies and core biopsies received for diagnosis in order to plan patient management.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.981305/full#supplementary-material

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Addressing quality and safety in anatomic pathology in low- and middle-income countries

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The World Health Organization (WHO) has created a sustainable development goal of reducing preventable mortality from cancer in low- and middle-income countries (LMICs) by 30% by 2030. Central to achieving this goal is the creation and maintenance of quality anatomic pathology services (APS). Within the last decade, quality assurance programs and patient safety measures have become a major focus of research for upper middle- and high-income countries (UMHICs), which has led to marked documented improvement in the quality of services provided by laboratories, as well as a decrease in patient safety events. We propose that as APS are developed in LMICs, the lessons learned by UMHICs are necessary to incorporate to produce quality and safe services toward obtaining the aforementioned goal. Furthermore, data suggests that Quality Improvement work requires change at the macrosystems and microsystems levels to achieve these goals. Here, we propose five "microsystems" strategies for professional organizations, healthcare institutions in LMICs and UMHICs that would accelerate quality improvement programs/systems implementation in APS in LMICs.

KEYWORDS

quality, anatomic pathology, LMIC (low- and middle-income countries), Africa, patient safety, quality assurance

1. Introduction

Cancer is the second leading cause of death from non-communicable diseases in low- and middle-income countries (LMICs). It is estimated that 75% of cancer deaths worldwide will occur in LMICs by 2030 (1). Achieving the World Health Organization (WHO)/World Bank sustainable development goal (SDG) of reducing preventable mortality from cancer in LMICs by 30% by 2030, requires that LMICs have reliable, consistent attention to a delivery structure for Anatomic Pathology Services (APS) (2).

Anatomic diagnosis is central to the diagnosis and treatment of malignancy at the individual patient level. Histopathologic evaluation assigns subtype, grade and microscopic stage, and identifies secondary prognostic features and molecular signals that predict prognosis and the choice of therapy for cancer. APS also play a critical role at the public health level for the prevention of cancer. Cervical cancer, for example, has been well-controlled in the upper middle- and high-income countries (UMHICs)

through pap smears—the single most successful prevention strategy for cancer, but which has defied implementation in LMICs (3). Cervical cancer prevention in LMICs is also limited by the fact that human papilloma virus (HPV) testing (and vaccines), now commonplace in North America, is too expensive for implementation in LMICs (4). Lastly, APS are critical to providing data for cancer registries and informing on the evolving epidemiologic patterns of cancer in a country to direct policy and resource allocation.

Pathology and Laboratory Medicine services, the broader group within which APS is situated, face substantial implementation challenges in LMICs (5). Wilson et al. identify four critical issues: (1) insufficient human capacity; (2) inadequate infrastructure; (3) inadequate education and training; and (4) inadequate quality, standards and accreditation. To date, laboratory services improvement efforts have necessarily focused on policies and processes of developing capacity and infrastructure at a macrosystems level (6).

However, as has become clear in the last decade, quality is essential and as much imperative as access to improve outcomes; poor quality is a bigger barrier to reducing mortality than access (7–9). Furthermore, evidence suggests that quality of care in extreme low resource and low resource settings, is challenged not, as was previously believed, by overwork, but by underwork, large know-do gaps and suboptimal context-level effective management (10). In addition, determinants of healthcare professional practice (organizational readiness, culture, and behavior of professionals) are critical to implementation success, and thus form integral components of implementation frameworks such as the Consolidated Framework for Implementation Research (CFIR) (11–13). Low resource organizational microsystems face similar organizational-cultural behaviors that create inefficiencies and result in ineffective and inconsistent processes which limit healthcare delivery in UMHICs (10, 14). Professional-cultural behaviors and resistance to change are among the greatest barriers to quality outcomes (14). This evidence suggests that delivering and implementing high quality outcomes requires a combined approach with efforts that are top-down, macro-mesosystems level and policy-regulatory, and microsystems-level, bottom-up and frontline organizational-institutional (10). Thus, even as countries work toward creating policy, regulatory, and infrastructure to improve access to APS, we suggest that microsystems "quality improvement and cultural change" work at the organizational levels needs to begin in LMICs to ensure that capacity and infrastructure investment deliver results (15). UMHICs need to partner with LMICs to jumpstart and accelerate this movement.

Effort has started with respect to microsystems quality improvement for laboratory medicine services, especially under the auspices of Strengthening Laboratory Management Toward Accreditation (SLMTA); it is primarily directed toward clinical laboratory services (16). However, APS requires a separate and particular consideration because of its positioning in diagnosis. The practice of diagnostic surgical pathology is higher risk—it is often the final word on cancer—one that overrules virtually all other tests; the tissue sample is an irretrievable, irreplaceable specimen. Moreover, APS professionals (pathology assistants, histotechnologists, cytotechnologists) and surgical pathologists require different training and skillset development, for a more manual laboratory structure, that requires a more hands-on-quality improvement methodologic knowledge base, compared to clinical laboratory medicine professionals.

Here, we propose five strategies to start a bottom up, microsystems level movement for quality improvement in LMICs, assisted by partners including colleagues, professional organizations and healthcare institutions in UMHICs. Implicit in these considerations are potential lessons for UMHICs to address waste, low-value care and disparities of care that plague some UMHICs.

2. Setting the expectations of diagnostic quality and accuracy begins with education: Education in quality and safety should be incorporated into all levels of healthcare and pathology curricula in LMICs

Although UMHICs have made significant strides in achieving impressive health outcomes, this has come at the cost of significant waste, and disparate outcomes especially for low resource and vulnerable populations. It is estimated that 30% of healthcare costs in the US are a waste from low-value care; a significant amount from unnecessary, repeat and wasteful testing (17). It is also estimated that there is significant delay to universal uptake of evidence-based practice guidelines even in UMHICs (18). To address these weaknesses in the healthcare delivery structure, the US has committed to quality in health with its six pillars—safe, timely, efficient, effective, equitable and patient centered care. To achieve this goal of quality and safety (Q and S) in healthcare, the Accreditation Council for Graduate Medical Education (ACGME) now requires dedicated Q and S training be part of residency training in the United States (19). The goal of this training is producing practitioners who can evaluate their own unsupervised practice and institute quality improvement measures for lifelong learning and delivery of high-quality care. An important reason for instituting this change at the trainee level is that professional cultural change is critical for a safety culture mindset, and habits are hard to break.

We propose that all APS training at all levels (technical through professional) in LMICs incorporate and require basic training in quality and safety. This is critical to empower local pathologists and laboratorians to use Evidence to Decision (EtD) frameworks to assess and transform UMHIC-developed protocols to local settings (20). A local *de jure* standard that

conforms to the highest level possible requires considerations of feasibility, cultural acceptance and balances benefits against harms (21). The blind adoption of specimen processing protocols from UMHICs, developed for patient populations where the primary presentation of cancer is early, where the harms-benefits equation differs, and where the choice of treatment interventions varies significantly will likely result in significant waste in an LMIC setting.

The SLMTA and Stepwise Laboratory Improvement Process Toward Accreditation (SLPTA) quality improvement programs have achieved significant gains in advancing quality and safety in clinical laboratories in LMICs (16, 22). However, APS-specific Q and S protocols are not part of this process. Q and S training is particularly important in APS as it is a more manual, less automated, process. Histochemical and immunohistochemical tests are laboratory-developed and require constant tweaking, monitoring and modification, using a structured quality improvement approach, to achieve optimal performance.

Basic "general medicine" Q and S training is available from various sources. We favor the Q and S modules from Institute of Healthcare Improvement, which offers a basic certification in Q and S for free for healthcare providers (23). While not APS specific, training in the basic principles of quality and safety ensures that medical professionals develop a mindset that is attentive to quality and begins the journey toward organizational and professional culture change, so that organizations achieve change readiness to reap the rewards of infrastructure investment. Data shows that even in low resource settings, a culture of safety is critical to optimizing outcomes (24, 25). Furthermore, the Model for Improvement (Plan-Do-Study-Act) is a model that is applicable at the microsystems level and allows for incremental quality improvement even in resource-limited settings (26). Every pathology department should train and designate an individual responsible for departmental quality and patient safety. Education in the basic principles of quality and safety will allow individuals in existing structures to not just optimize outcomes but identify and develop quality metrics that are context specific.

Anatomic Pathology Q and S education modules are in development as part of the Open Pathology Education Network (OPEN) project, which aims to develop a digital pathology enabled virtual curriculum to improve the global pathology workforce, using the DPA-DAPA (Digital Pathology Association and Digital Anatomic Pathology Academy) resources (27–30).

3. Develop resource-stratified external quality assurance and quality improvement collaboratives for LMIC regions

Laboratories in similar settings face similar challenges and can benefit from each other. Project ECHO (Extension for Community Health Outcomes), from the University of New Mexico, is an "all teach, all learn" education and care management model, that "moves knowledge, not people" (31). It is a model based in virtually connecting healthcare professionals to experts to address care-management problems and improve patient outcomes. The model has been successful in providing consultant and expert support to peripherally located generalist clinicians, remote oversight of tumor boards, and support to teaching hospitals in LMICs. The model has been successfully leveraged in at least two settings with respect to laboratory medicine and APS. In one model, pathologists participate in providing tumor-board support to International Gynecological Cancer Society supported gynecological oncology training programs in LMICs (32). The Division of Laboratory Systems, Centers for Disease Control and Prevention (CDC) successfully piloted a laboratory community of practice (CoP) on diagnostic excellence using an adaptation of the clinician-based ECHO Model. The project aims were to (1) connect laboratory professionals, clinicians, and subject matter experts in laboratory medicine, (2) use case-based learning to educate and train laboratory and healthcare professionals, and (3) examine cross-cutting issues in clinical and anatomic pathology (33). Serendipitously, this project was ongoing during the recent COVID-19 pandemic and facilitated transfer of knowledge and lessons learned from frontline states to other states and allowed them to prepare somewhat for the onslaught of the coming pandemic (34).

Extension for Community Health Outcomes-like models, combined with telepathology, are high potential for APS-efforts for diagnostic quality improvement and education, especially as the COVID pandemic has accelerated the adoption of digital diagnostic APS in UMHICs (35). To date, telepathology efforts have been used to provide diagnostic support from a UMHIC collaborator to an LMIC site (36). A small External Quality Assurance (EQA) in diagnosis for APS for LMICs (Ghana and Nigeria) hosted by Leeds University is also reported (37) (the authors were unable to access the further data about this program). However, there is likely a need to develop LMIC context-specific EQAs as the use of EQAs in UMHICs may not necessarily improve implementation in LMICs.

Quality improvement collaboratives (QICs) aim to bring together institutions (laboratories) to implement evidence-based quality improvement initiatives. These have been used in a variety of specialties, often across geographic borders. They have been instrumental in improving infectious disease care (particularly tuberculosis and human immunodeficiency virus care) (36). The existing structures are now being leveraged to expand the initiatives to non-infectious diseases with the hopes of accelerating the time to achieving the non-infectious disease SDGs (38). To our knowledge, QICs have not been used in APS.

One of the authors (VP) is attempting start one such initiative for extreme low resource settings where even low-resource models are untenable (39). She is among a small

group of pathologists who is participating in a pilot to provide remote pathology services at ECWA Hospital, Egbe, Nigeria. The program is modeled on a successful grass-roots effort at the Mbingo Baptist Hospital Pathology program in Cameroon. A relative low-cost ($10,000 USD) manual telepathology set up from Microvisoneer^{TM}[1] allows pathologists in the US to provide diagnostic services; a similar project has been started in Djibouti (40).

4. Development of resource-stratified stage-based guidelines for pathologic assessment and reporting of malignancy should be a priority for achieving an appropriate standard of care in LMICs

Pathologic evaluation protocols that are high quality and provide accurate diagnoses and information allow for timely and appropriate intervention. With rapid advancements in technology, molecular characterization of tumors has become essential to subtype tumors and to allow for targeted therapies in UMHICs. However, this diagnostic standard has the potential to increase the gap in diagnostic accuracy between UMHICs and LMICs, because the diagnostic criterion is the molecular signature (e.g., the diagnosis of partial mole requires the demonstration of diandric triploidy). While this progress should not be curtailed or decelerated in UMHICs, especially as it will eventually benefit patients even in LMICs, there is need to develop evidence-based protocols that are resource-stratified.

The key standard is an evidence-based, stage and resource-stratified guideline for pathologic cancer assessment and reporting. This is needed both at the gross and diagnostic level as a standard of care that is insensitive to implementation factors will necessarily fail.

Grossing protocols developed in UMHICs where malignancy presents early are likely to be wasteful in LMICs. The two most important predictors of outcome for cancer are histologic type and stage. Considering that cancers in LMICs not infrequently present at advanced stages (secondary to lack of screening availability more commonly seen in UMHICs), the extensive blocking-grossing protocols of UMHICs add cost without value in LMICs. Stage-stratified grossing protocols are one possible solution and may help reduce waste even in UMHICs, as current grossing protocols in UMHICs are independent of stage parameters. The incremental value from expansive sectioning of the ovary and uterus, in the setting of widely metastatic peritoneal carcinoma is unclear. A more cost-effective strategy may be to submit 2–3 sections of the metastatic tumor for diagnosis. Similarly, detailed subtyping of tumors (determining serous carcinoma vs. high grade endometrioid carcinoma) may not be value-added, if no treatment differences exist in a resource-limited setting.

Clinical societies have already adopted this approach and have partnered with clinical domain experts in Sub-Saharan Africa to propose alternative pathologic evaluation guidelines for resource limited settings. However, at least in some cases, these are incongruous as they are created without knowledge of pathology processing (41). Thus NCCN guidelines suggest that ultrastaging may not be feasible in low-resource settings, but recommend reporting of isolated tumor cells and micrometastases, which necessarily require ultrastaging (42). Some ignore more simplistic and efficient strategies to obtain relevant information. The direct involvement of surgical pathologists in the creation of such guidelines is critical, especially from those with experience or expertise in resource-limited settings. Involvement of experts from developing countries (e.g., India) may be particularly beneficial as they may bridge the gap between extreme low resource and high resource settings and may offer expertise that has been lost in UMHICs. A challenge faced by one of the authors when participating in a cervical cancer grassroots effort in South India was that few cytologists or cytopathologists in the US felt competent to read routine pap smears; they had all transitioned to or only trained in liquid based cytology.

5. Academic institutions associated with UMHICs should expand affiliations to include pathology at outreach facilities in LMICs

Many academic medical centers in UMHICs have affiliate programs at universities in LMICs, to enrich educational experiences in infectious disease and low-resource primary-care rotations for trainees (43). This infrastructure can be leveraged to build bridges between pathology departments in UMHICs and LMICs. Indeed, two of the most successful UHMIC-LMIC pathology programs—one in Rwanda and the other in Malawi were developed by leveraging existing connections for infectious diseases (44, 45). This structure should also allow for exchange of knowledge for better diagnostic accuracy. In general, points for consideration would include:

1. Leverage telepathology to offer consultations and more to affiliates in LMICs.
2. Create structured bilateral exchange programs for short-term rotation for visiting pathologists and laboratory

1 https://www.microvisioneer.com/

professionals between affiliate UMHIC and LMIC academic programs.

3. Develop mechanisms by which affiliate pathologists in LMICs can attend educational, clinical and Q and S conferences in UMHIC. COVID created pressure for educational programs in UMHICs to move to hybrid learning modules (45). This offers unprecedented opportunity to include trainees and pathologists in LMICs in educational offerings without added costs to either site. We hope that UMHICs will consider partnering with pathology programs in LMIC for improved education and training within existing structures. The development of collaborative educational efforts also enriches the educational experiences of trainees in UMHICs, giving them a perspective of global health issues. This also helps to develop relationships between trainees across disparate settings that could sustain through their careers and help strengthen bridges between pathology programs in LMICs and UMHICs.
4. An eventual goal maybe to develop primarily on-line training models for LMICs, especially for training laboratory professionals.

6. Engagement of the international medical graduate diaspora in UMHICs for the education of and development of LMIC pathology services

Pathology services in UMHICs are provided to significant extent by providers who have roots in LMICs. In 2022, 45% of entry level trainees in the US in pathology were international medical graduates (IMGs) (46); a significant plurality from LMICs. There is anecdotal evidence that many IMGs—particularly later in life, after achieving financial and child-rearing obligations— look to contribute to advancing healthcare in the LMIC country of their roots. This connection could be tapped and encouraged by institutions in UMHICs for collaborative efforts and support for LMICs.

Current reward structures in UMHIC academic institutions value publications in prestigious journals, typically of studies with molecular inquiry of disease, and presentations at UMHIC national meetings or at institutions, while discounting public health and educational efforts in LMICs for academic advancement. Service on certain types of committees in professional societies in UMHICs is also valued over involvement in less visible grass-roots efforts in low-resource settings. Recognition of work in LMICs as a value-added effort rather than simple volunteerism could incentivize greater involvement in LMIC work from IMGs in UMHICs. Furthermore, professional societies should look to this untapped resource for LMIC work as these individuals have cultural, language and context specific competencies to move LMIC programs forward more quickly.

7. Conclusion

In summary, improving the quality of APS in LMICs is essential to achieving SDG. While significant effort is still needed from countries at the policy and infrastructure levels, some changes, including education in quality improvement at the microsystems levels (institutional and organizational levels) within LMICs will allow for improved quality and optimization of existing diagnostic services and create organizational readiness and cultural change to optimize returns from infrastructure investments. These investments, while small, have the potential, to allow for adoption and adaptation of evidence-based models to local constraints and identify novel strategies for success in low resource settings.

Academic institutions in UMHICs need to invest in establishing foundational and sustainable educational and clinical frameworks to promote quality pathology services in LMICs. Changing incentive and advancement reward structures in UMHICs academic institutions can promote greater investment from the IMG diaspora in LMIC work. Professional organizations need to commit more toward these efforts and engage the IMG diaspora in pathology in LMIC work; the IMG diaspora offers a ready resource that remains untapped. These small but important changes have the potential to help the world meet SDG for cancer.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

VP, SS, and AE were involved in the creation, drafting, and revising of this manuscript. All authors agree with the manuscript as submitted herein.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Pattern and trends of *Helicobacter pylori* genotypes in gastric cancer: A Kenyan 8-year study

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Background: Notable geographic and temporal variations in the prevalence and genotypes of *Helicobacter pylori*, in relation to gastric pathologies, have been observed; however, their significance and trends in African populations is scarcely described. The aim of this study, was to investigate the association of *H. pylori* and its respective *CagA* and vacuolating cytotoxin A (*VacA*) genotypes with gastric adenocarcinoma, and to describe the trends of *H. pylori* genotypes over an 8-year period (2012–2019).

Materials and methods: A total of 286 samples of gastric cancer cases and benign controls (one-to-one matching), from three main cities in Kenya, between 2012 and 2019 were included. Histologic evaluation, and *CagA* and *VacA* genotyping using PCR, was performed. Distribution of *H. pylori* genotypes was presented in proportions. To determine association, a univariate analysis was conducted using a Wilcoxon rank sum test for continuous variables, and a Chi-squared test or Fisher's exact test for categorical data.

Results: The *VacA s1m1* genotype was associated with gastric adenocarcinoma, {odds ratio (OR) = 2.68 [confidence interval (CI) of 95%: 0.83–8.65]; $p = 0.108$}, whilst *VacA s2m2* was associated with a reduced probability of gastric adenocarcinoma [OR = 0.23 (CI 95%: 0.07–0.78); $p = 0.031$]. No association between cytotoxin associated gene A (*CagA*) and gastric adenocarcinoma was observed.

Conclusion: Over the study period, an increase in all genotypes of *H. pylori* was seen, and although no predominant genotype was noted, there was significant year-to-year variation, with *VacA s1* and *VacA s2* showing the greatest variation. *VacA s1m1* and *VacA s2m2* were associated with increased, and reduced risk of gastric cancer, respectively. Intestinal metaplasia and atrophic gastritis did not appear to be significant in this population.

KEYWORDS

***H. pylori* (*Helicobacter pylori*), genotype, gastric cancer, Kenya, trends**

Introduction

Helicobacter pylori is likely the most genetically diverse bacterial species and the most prevalent human pathogen (1, 2). This organism colonizes the stomach of approximately half of the world's population, and is etiologically associated with a wide spectrum of diseases, ranging from chronic gastritis, to peptic ulcer disease, and gastric adenocarcinoma (2, 3).

A recent systematic review and meta-analysis on the global prevalence of *H. pylori* infection, found that Africa had the highest pooled prevalence (70.1%) whilst Oceania and North America had the lowest (24.4%) (2). This study included works published from 62 countries between 1970 and 2016, using heterogeneous modalities of detection, including, serology, stool antigen test, urea breath test, culture and histopathology. Locally, a Kenyan study sampling 487 patients with dyspepsia, and utilizing rapid urease, histopathology and culture tests, found the prevalence to be 40.86%, lower in comparison to reports from other developing countries (4).

The GLOBOCAN 2020 report, listed gastric cancer as the fifth most common cancer worldwide, with over one million new cases reported, more than 70% of these found in developing countries. In addition to this, it was found to be the fourth leading cause of cancer-related mortality worldwide, in both sexes, accounting for nearly 769,000 deaths (i.e., 7.7% of all new cancer related deaths) (5); and interestingly, African patients were found to present not only at a younger age (between the third and the fourth decade), but also a more advanced stage of disease (6).

The majority of gastric cancers are sporadic, with chronic *H. pylori* infection found to be the main risk factor in up to 90% of gastric cancer cases (5). Studies have shown a sixfold increase in the risk of gastric cancer among *H. pylori* infected populations, in comparison to the uninfected (1), and as such, it has been classified as a type 1 carcinogen by the International Agency for Research on Cancer (IARC), WHO (7).

The fact that only a subset of infected people develop severe gastrointestinal disease has been attributed to several factors related to the host, environment and bacterium (3). With reference to the bacterium, cytotoxin associated gene A (*CagA*) and the vacuolating cytotoxin A (*VacA*), appear to be the major virulence factors involved in disease pathogenicity, in an allele-dependent manner, potentially explaining the global geographic distribution of gastric adenocarcinoma (8). The meta-analysis by Pormohammad et al. determined that these molecules are significantly associated with an increased risk of gastric cancer, with an odds ratio (OR) to detect of 2.82 and 1.75 for *CagA* and *VacA*, respectively. Moreover, the prevalence of the *CagA* gene in gastric adenocarcinoma was found to be 74%, with the *VacA s1m1* mosaic combination identified in 52.4% of cases (9). A local study investigating the association between *H. pylori* and clinical outcomes, found that *H. pylori* was detectable in 62.9% of 127 patients with dyspepsia. Of the 127 patients, only 10 had a diagnosis of gastric cancer. The prevalence of the *CagA* gene was found to be 48.75%, with no significant association with gastrointestinal disease, including cancer, and in contrast, the *m2*, *i2*, and *s2* alleles of the *VacA* gene were found in 65, 52, and 49%, respectively, with varying but significant associations with gastric cancer (10).

Despite having the highest pooled prevalence of *H. pylori*, the rates of gastric cancer in Africa remain among the lowest worldwide. In Eastern Africa for example, the rates are 4.9 and 4.2 per 100,000 in men and women, respectively, whilst in Eastern Asia, the rates are 32.5 and 13.2 per 100,000 in men and women, respectively (5). In 2020, the incidence of gastric cancer in Kenya was 7.2 per 100,000 in the male population, and 7.7 per 100,000 in the female population, with a mortality rate of more than 6.6 per 100,000 (5). The prevalence in Nairobi, Kenya, according to the only available data from a population-based cancer registry between 2004 and 2008, was 6.2% (11); however, it is believed that this may be higher given the GLOBOCAN estimated incidence rates for the Eastern Africa region. Different tumor biology, and perhaps an overestimation of the *H. pylori* prevalence may confound the actual situation (2, 6); however, due to a paucity of data, particularly in African populations, this cannot be commented on.

Cancer continues to show an upward trend, more so in developing countries. As pertains specifically to gastric adenocarcinoma, the knowledge of the distribution and significance of *H. pylori* is not only scant, but remains inadequately investigated in African populations, particularly in Kenya. Furthermore, the *H. pylori* genotypes not only vary from one region to the next, but also show variation over time. As such, it is necessary to obtain more up-to-date data within any given population, with the aim of expanding the knowledge of the role, and magnitude this bacterium plays in the gastric cancer burden (2). Ultimately, stratification of persons at increased risk of developing gastric cancer, and management strategies that involve *H. pylori* eradication programs and follow-up, can be rationalized based upon the findings of such studies.

Thus, the objectives of this study are to describe the association of *H. pylori* and its respective *CagA* and *VacA* cytotoxic genes with gastric adenocarcinoma and, to describe the trends of *H. pylori* genotypes over the last 8 years (2012–2019) in Kenya.

Materials and methods

Study design and setting

A case-control laboratory-based study was designed including three study sites; The Aga Khan University Hospital Nairobi (AKUHN), and The Aga Khan Hospitals (AKH) Mombasa, and Kisumu. These institutions are located in the three main cities in Kenya, and represent cosmopolitan populations.

Sample size and sampling process

Expecting that the age and the sex group might be determinant for both the histological lesion and the presence of *H. pylori*, consecutive samples of gastric cancer cases reported between 2012 and 2019 were matched randomly from a pool of benign gastric biopsies in a one-to-one process. With a minimum OR to detect of two (based on the meta-analysis by Pormohammad et al.), and a projected power of greater than 80%, the expected minimum number of cases was 143, combined from all three

facilities, with an equal number of controls and hence a total sample size of 286. All samples were de-identified formalin fixed paraffin embedded (FFPE) tissue blocks, and, were either biopsy, excision or gastrectomy specimens. Samples with insufficient material were excluded, whilst only those with biodata (age and sex) were included.

Laboratory procedures

Sectioning of FFPE blocks

Selected FFPE blocks were sectioned for both histological assessment and molecular analysis of *H. pylori*, *VacA* and *CagA* genes. The first sections were cut at 3 μm for standard haematoxylin and eosin (H&E) staining. This was followed by five sections of 6–8 μm each, placed in 2 ml micro centrifuge tubes for DNA extraction. To avoid carry-over contamination, a new blade was used for each FFPE block, and the microtome overlay cleaned after each case with DNA cleaner. As a quality control measure to monitor effectiveness of carry-over contamination, prevention procedures were conducted using a blank paraffin block sectioned after every 10 cases and processed for PCR alongside the other cases.

Histology

Standard H&E staining was performed on 3 μm sections of all FFPE blocks of the cases and controls on the Dako autostainer platform (Dako, Denmark). Joint histologic review of all the cases and controls using a consensus approach by two registered pathologists and one resident was performed. Gastric cancer was classified using the Lauren classification, while gastritis using the Sydney system. The presence of *H. pylori* was assessed on newly stained H&E and original Giemsa sections, and was reported as positive if identified on either stain.

Isolation and purification of DNA

Total genomic DNA was extracted from the tissue sections as per the manufacturer's protocol using a commercial kit (QIAamp DNA FFPE tissue kit, Qiagen, Hilden, Germany). Following dewaxing, which involved two washes in xylene and two washes in absolute ethanol, the tissue pellets were suspended in 180 μL of ATL buffer and 20 μL of proteinase K. These were incubated at 56°C until completely lysed, and then at 90°C for 1 h, followed by addition of 200 μL of AL buffer and 200 μL of ethanol (99.5%), prior to purification through QIAamp spin columns. Extracted DNA was concentrated using DNA Clean & Concentrator-100 (Edge Bio's Performa® DTR Gel Filtration Cartridges, San Jose, CA, USA) following the manufacturer's protocol.

CagA and *VacA* genotyping

Amplification and genotyping of the *Cag* and *Vac A*, *s*, and *m* regions was performed using end-point PCR, with previously published PCR conditions from similar studies referred to (12). To confirm the presence of *CagA* gene, we corrected a set of nucleotide primers previously described (13), based on sequence variations noted in the Kenyan *H. pylori* isolates (14), as well as other African isolates retrieved from.[1] This process was performed using the Qiagen CLC genomics workbench, and the following forward and reverse primers selected: *CagA*-CA-OMF: 5′-CAA GCA AAA AGC GAC CTT GAA A-3′ and *CagA*-Ke-OMR: 5′-ACA CCA TTC TTA ACG GAT TG-3′(248 bp). The primers used for *VacA* s_1/s_2 were VA1-F 5′- ATGGAAATACAACAAACACAC-3′, and VA1-R 5′-CTGCTTGAATGCGCCAAAC-3′ (product size 259/286 bp); and, the primers used for *VacA* m_1/m_2 were, VAG-F 5′-CAATCTGTCCAATCAAGCGAG-3′ and VAG-R 5′-GCGTCAAAATAATTCCAAGG-3′ (product size 570/645 bp). The specificity of the primer set was assessed using the Blast search through the NCBI database.[2] Amplification was achieved by an initial denaturation step of 10 s at 98°C, followed by 30 cycles of 10 s at 98°C, 30 s at 55°C and 20 s at 72°C; and a final extension step of 1 min at 72°C. Each PCR reaction (12.5 μL), contained 6.25 μL premix (Takara Emerald Amp® Max PCR Master mix, Kusatsu, Japan), 2 μL of forward and reverse primers each and 2.25 μL of purified DNA. The PCR products were electrophoresed using Agarose gel (Nippon gene Agarose S, Tokyo, Japan) at 2 g/100 ml, with ethidium bromide concentration of 3 μL/100 ml at a voltage of 135 volts, for 35 min.

Reference samples

As reference samples for the assessment of *Vac s* and *m* region, *H. pylori* Tx-30a (ATCC 51932) (*CagPAI* negative, s_2m_2) and *H. pylori* 26695 (*CagA* positive, s_1m_1) were cultured and used as positive controls for the experiment. Similarly, for *CagA*, Kenyan isolate 78 (*CagA* positive, s_1m_1) and *H. pylori* 26695 (*CagA* positive, s_1m_1). These isolates were inoculated under a biological safety cabinet onto a *Helicobacter* selective Agar medium (Nissui Pharmaceutical co., Ltd., Tokyo, Japan) and incubated for 10 days. The colonies growing on the plates were identified and sub cultured for 3–4 days at 37°C in microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2). Sub culture was on Brucella Agar plates (Becton Dickinson, Sparks, MD, USA), supplemented with 7% horse serum (Nippon Biotest Laboratories Inc., Tokyo, Japan). The colonies were identified as small, round, translucent and the organisms were gram-negative and positive for the urease test.

Statistical analyses

Baseline characteristics, histological and molecular data for each sample were entered into a Microsoft Excel database. The results for each of the target genes were recorded in a binary format (i.e., absent or present for both the case and control groups); and compared using a 2 × 2 contingency table. A univariate analysis was conducted using a Wilcoxon rank sum test for continuous variables and a Chi-squared test or Fisher's exact test for categorical data, when comparing the cases and controls. The homogeneity of variances and normality of distributions were assessed using the Levene and Shapiro–Wilk tests. Odds ratios (ORs) were used to measure the association between qualitative variables and a *p*-value of less than 0.05 was considered statistically significant.

1 https://ncbi.nlm.nih.gov

2 https://blast.ncbi.nlm.nih.gov

Ethical considerations

This study was conducted after ethical approval from The Aga Khan University, Institutional Ethics Review Committee (IERC), (reference 2019/REC-40). Under this approval, only de-identified archival tissues were used.

Results

Baseline characteristics

Overall, 286 samples comprising 143 gastric cancer cases and an equal number of age, and sex matched controls were analyzed (Supplementary Table 1). The age range was between 21 and 92 years, with the mean and median ages found to be 61 years on both accounts. The highest frequency of age distribution (51.7%) was found to be in patients equal to or greater than 60 years; with samples from patients between the 5 and 6th decades accounting for 37.1% (53/143), and those between 21 and 40 years accounting for 11.2% (16/143). The male to female ratio of gastric cancer in this sample was 1.5:1. For the majority, (53.1%) the location of the cancer was not specified, 8.9% (27/143) were from the gastric antrum and 16.8% (24/143) from the gastroesophageal junction (GEJ), 4.2% each from the body and fundus, and 2.8% from the cardia. The control samples had a nearly similar distribution; however, there were larger numbers from the gastric antrum and body compared to the GEJ. Ultimately, the contribution of samples from AKH Mombasa was too small (1.4%) for any accurate assessment of *H. pylori* genotypes in that city to be conducted (Table 1).

TABLE 1 Baseline characteristics of the samples analyzed.

Characteristics	Cases $n = 143$ (%)	Controls $n = 143$ (%)	All samples $n = 286$ (%)
Age group			
(21–40)	16 (11.2)	16 (11.2)	32 (11.2)
(41–60)	53 (37.1)	53 (37.1)	106 (37.1)
(61–80)	66 (46.2)	66 (46.2)	132 (46.2)
> 80	8 (5.6)	8 (5.6)	16 (5.6)
Gender			
Female	58 (40.6)	58 (40.6)	116 (40.6)
Male	85 (59.4)	85 (59.4)	170 (59.4)
Study sites			
AKH_Kisumu	32 (22.4)	32 (22.4)	64 (22.4)
AKH_Mombasa	2 (1.4)	2 (1.4)	4 (1.4)
AKUH_Nairobi	109 (76.2)	109 (76.2)	218 (76.2)
Type of samples			
Biopsies	134 (93.7)	143 (100)	277 (96.9)
Excisions	9 (6.3)	0 (0)	9 (3.1)
Site of sample collection			
Antrum	27 (18.9)	56 (39.2)	83 (29.0)
Cardia	4 (2.8)	0 (0.0)	4 (1.4)
GEJ	24 (16.8)	1 (0.7)	25 (8.7)
Body	6 (4.2)	23 (16.1)	29 (10.1)
Fundus	6 (4.2)	2 (1.4)	8 (2.8)
Gastric, site not specified	76 (53.1)	61 (42.7)	137 (48)

Histologic features and *H. pylori* genotypes in gastric adenocarcinoma

As depicted in Figure 1 the majority of the gastric adenocarcinoma cases, were of the intestinal type, 61.5% (88/143). The diffuse, mixed and indeterminate types accounted for 25.9% (37/143), 7.7% (11/143), and 4.9% (7/143), respectively. A total of 46 (32.1%) gastric adenocarcinoma cases were found to be positive for *H. pylori* on both molecular and histologic analyses, with the error of double counting circumvented, and 97 (67.8%) were *H. pylori* negative.

Out of the 46 *H. pylori* positive cases, 67.4% (31/46) were of the intestinal type, while the diffuse, mixed and indeterminate types accounted for 19.6, 8.7, and 4.3%, respectively. The majority of the *H. pylori* positive gastric cases were found to be poorly differentiated, 60.9% (28/46), while moderately and well-differentiated cases accounted for 37% (17/46) and 2.2% (1/46), respectively (Figure 1).

Further *H. pylori* genotype analysis was successful in only 38 of the *H. pylori* positive cases. These included 27 cases of the intestinal subtype, eight of the diffuse, and two of the mixed type, with only one indeterminate case. Specific to the intestinal type, the *CagA* genotype was identified in 48.1% (13/27) of the *H. pylori* positive cases, with slightly over a half, 51.9% (14/27), found to be negative. *VacA s1* genotype was identified in 14 cases (51.9%) and *VacA s2* in 11 cases (40.7%), while *VacA m1* and *m2* were encoded in 55.6% (15/27) and 33.3% (9/27) accordingly. The predominant mosaic combination was *VacA s1/m1*, with a frequency of 33.3% (9/27) followed by *s1/m2*, 14.8% (4/27). The other genotypes, *s2/m1* and *s2/m2*, were identified in 5 (18.5%) and 4 (14.8%) cases, respectively. Further to the *H. pylori* positive diffuse-variant cases, 75.0% (6/8) were positive for *CagA*. *s1* and *s2 Vac* regions were encoded in the following frequencies, 87.5% (7/8) and 25.0% (2/8); with *m1* and *m2* found to be present in 75.0% (6/8) and 37.5% (3/8), respectively. The predominant mosaic combination was once again *VacA s1/m1* with a frequency of 62.5% (5/8). *s1/m2* was detected in 2 cases (25.0%), and of the remaining genotype combinations, only *s2/m1* was recorded (11.1%) (Table 2).

The commonest pathology amongst the controls was chronic gastritis, 60.8% (87/143), with activity (neutrophil infiltrate) present in 43.7% of these. Biopsies exhibiting no pathology accounted for 28.7% (41/143), and other forms of gastritis (acute, atrophic and reactive) formed the remainder of the samples. Only 43 of the 143 controls tested positive for *H. pylori*, with the error of double counting circumvented. The majority of these were chronic gastritis (76.7%). Intestinal metaplasia and atrophic

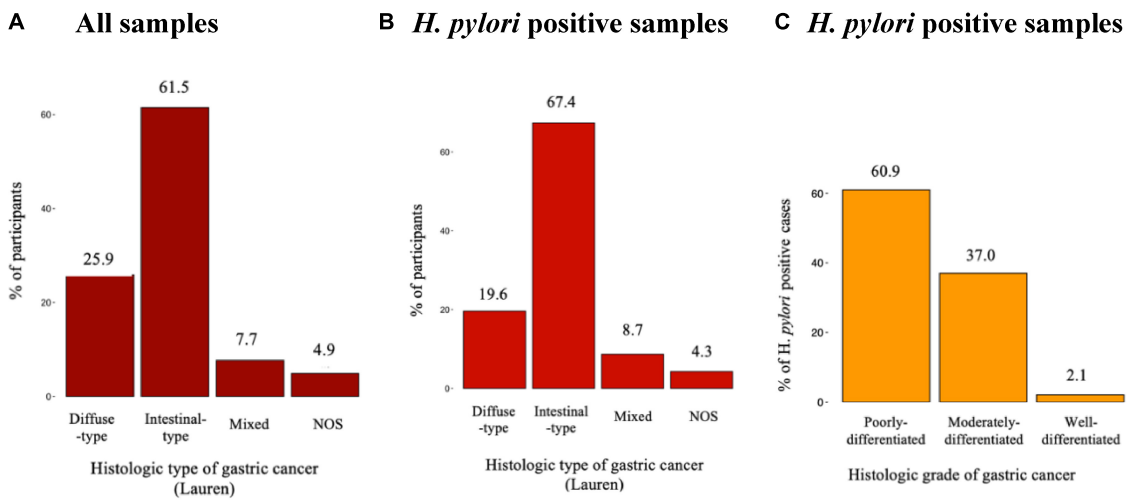

FIGURE 1
Distribution of *Helicobacter pylori* amongst the histologic types and grades of gastric adenocarcinoma. **(A)** All samples, **(B)** *H. pylori* positive samples, and **(C)** *H. pylori* positive samples.

TABLE 2 *Helicobacter pylori* genotype distribution amongst the histologic subtypes of gastric adenocarcinoma.

H. pylori genotype	Intestinal-type $n = 27$ (%)	Diffuse-type $n = 8$ (%)	Mixed $n = 2$ (%)	Indeterminate $n = 1$ (%)
CagA positive	13 (48.1)	6 (75.0)	0 (0.0)	0 (0.0)
VacA s1	14 (51.9)	7 (87.5)	1 (50.0)	0 (0.0)
VacA s2	11 (40.7)	2 (25.0)	1 (50.0)	1 (100.0)
VacA m1	15 (55.6)	6 (75.0)	1 (50.0)	0 (0.0)
VacA m2	9 (33.3)	3 (37.5)	0 (0.0)	1 (100.0)
VacA s-m1	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)
VacA s-m2	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)
VacA s1m1	9 (33.3)	5 (62.5)	0 (0.0)	0 (0.0)
VacA s1m-	1 (3.7)	0 (0.0)	1 (50.0)	0 (0.0)
VacA s1m2	4 (14.8)	2 (25.0)	0 (0.0)	0 (0.0)
VacA s2m1	5 (18.5)	1 (12.5)	1 (50.0)	0 (0.0)
VacA s2m-	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)
VacA s2m2	4 (14.8)	0 (0.0)	0 (0.0)	1 (100.0)

gastritis were found to be rare, 0.07% (10/143) and 0.06% (8/143), respectively.

Virulence and host gene detection in *H. pylori* cases and controls

In this study, the presence of the *VacA s2m2* genotype was significantly associated with a reduced probability of having gastric adenocarcinoma {OR = 0.23 [confidence interval (CI) 95%: 0.07–0.78]; $p = 0.031$}. In contrast, the *VacA s1m1* genotype was associated with gastric adenocarcinoma, however, this association was not statistically significant [OR = 2.68 (CI 95%: 0.83–8.65); $p = 0.108$]. There was no significant association observed between the *CagA* genotype and the gastric adenocarcinoma [OR = 0.69 (CI 95%: 0.25–1.87); $p = 0.628$] (Table 3).

Trends of *H. pylori* genotype expression over the study duration

Analysis of the trends of *H. pylori* genotypes over the 8 years, 2012 through 2019, was done using the 66 samples where molecular characterization was successful. This represented 23% of the entire sample size, and comprised 38 cases and 28 controls. Because of the small number in each year, two consecutive years were combined to create four time points. Genotypes with the greatest year-to-year variation as measured by percent coefficient of variation (%CV) were *VacA s1* and *VacA s2*, (%CV of 41 and 42, respectively). In contrast, *VacA m1* and *VacA m2* exhibited minimal variation (%CV of 4 and 11, respectively). The *CagA* genotype also showed marked variation (%CV 19). The trends are visualized in Figure 2.

TABLE 3 Detection and distribution of *H. pylori* virulence genes in cases and controls.

Genotype		OR (CI 95%)	Cases	Controls	*p*-value
			n = 143 (%)	*n* = 143 (%)	
CagA	Positive	0.69 (0.25, 1.87)	21 (55.2)	18 (64.3)	0.628
	Negative		17 (44.7)	10 (35.7)	
VacA m1	Positive	1.59 (0.59, 4.24)	22 (57.9)	13 (46.4)	0.501
	Negative		16 (42.1)	15 (53.6)	
VacA m2	Positive	0.39 (0.14, 1.07)	13 (34.2)	16 (57.1)	0.109
	Negative		25 (65.8)	12 (42.9)	
VacA s1	Positive	2.90 (1.05, 8.06)	22 (57.9)	9 (32.1)	0.068
	Negative		16 (42.1)	19 (67.9)	
VacA s2	Positive	0.42 (0.16, 1.15)	15 (39.5)	17 (60.7)	0.145
	Negative		23 (60.5)	11 (39.3)	
VacA s-[a]*m1*	Positive	0.23 (0.02, 2.29)	1 (2.6)	3 (10.7)	0.304
	Negative		37 (97.4)	25 (89.3)	
VacA s-m2	Positive	–	1 (2.6)	0 (0.0)	1.000
	Negative		37 (97.4)	28 (100.0)	
VacA s1m1	Positive	2.68 (0.83, 8.65)	14 (36.8)	5 (17.9)	0.108
	Negative		24 (63.2)	23 (82.1)	
VacA s1m-[b]	Positive	–	2 (5.3)	0 (0.0)	0.504
	Negative		36 (94.7)	28 (100.0)	
VacA s1m2	Positive	1.12 (0.29, 4.43)	6 (15.9)	4 (14.3)	1.000
	Negative		32 (84.2)	24 (85.7)	
VacA s2m1	Positive	1.04 (0.29, 3.69)	7 (18.4)	5 (17.9)	1.000
	Negative		31 (81.6)	23 (82.1)	
VacA s2m-	Positive	–	2 (5.3)	0 (0.0)	0.504
	Negative		36 (94.7)	28 (100.0)	
VacA s2m2	Positive	0.23 (0.07, 0.78)	5 (13.2)	11 (39.3)	0.031
	Negative		33 (86.8)	17 (60.7)	

[a]The *s* genotype is unknown because it was not detected. [b]The *m* genotype is unknown because it was not detected.

Discussion

Gastric cancer is the fifth most common malignancy, and the fourth leading cause of cancer-related deaths worldwide (5). On average, only 1–3% occur as part of a hereditary syndrome, with the greater majority of reported cases found to be sporadic, in which *H. pylori* infection has been established as the most important risk factor. As a strategy of mitigating gastric cancer risk therefore, both developed and low-and-middle income countries (LMIC) have instituted various *H. pylori* eradication programs. However, in spite of this fact, the latest GLOBOCAN report of 2020, evidenced an upsurge in gastric cancer cases, 70% of which were attributed to developing countries (5). This current study sought to evaluate the association of this bacterium and its virulence factors *CagA* and *VacA*, with gastric adenocarcinoma, and to describe the *H. pylori* genotype distribution in Kenya.

Similar to the global pattern, the rate of gastric cancer was higher in males than in females (ratio = 1.5:1). The median age at diagnosis was 61 years, and so, in contrast to previous studies, this study demonstrated a higher frequency in the elderly, rather than the previously reported third and fourth decade in African populations (6). Nevertheless, a significant proportion (11%), of gastric cancer occurred between the third and fourth decades, with the youngest patient being 21 years. These observations may be attributed to host and environmental risk factors, not evaluated in this study.

Concordant with the results of various gastric adenocarcinoma focused studies, intestinal variant was the most common (61.5%) followed by diffuse and mixed types (25.9 and 7.7%, respectively). Interestingly, however, these findings differed with a recently published study on gastric cancer in Kenya, where the authors found diffuse-type gastric cancer to be more common (15).

A total of 32% of the cases were found to be positive for the *H. pylori*, which was in contrast to a previously conducted local study in 2010, that demonstrated a prevalence of 0.9% (10). Further to this, more than half of the cases lacked a specified tumor site, 16.8% were from the GEJ and only 18.9% from the antrum. Given that the highest association with *H. pylori* is with

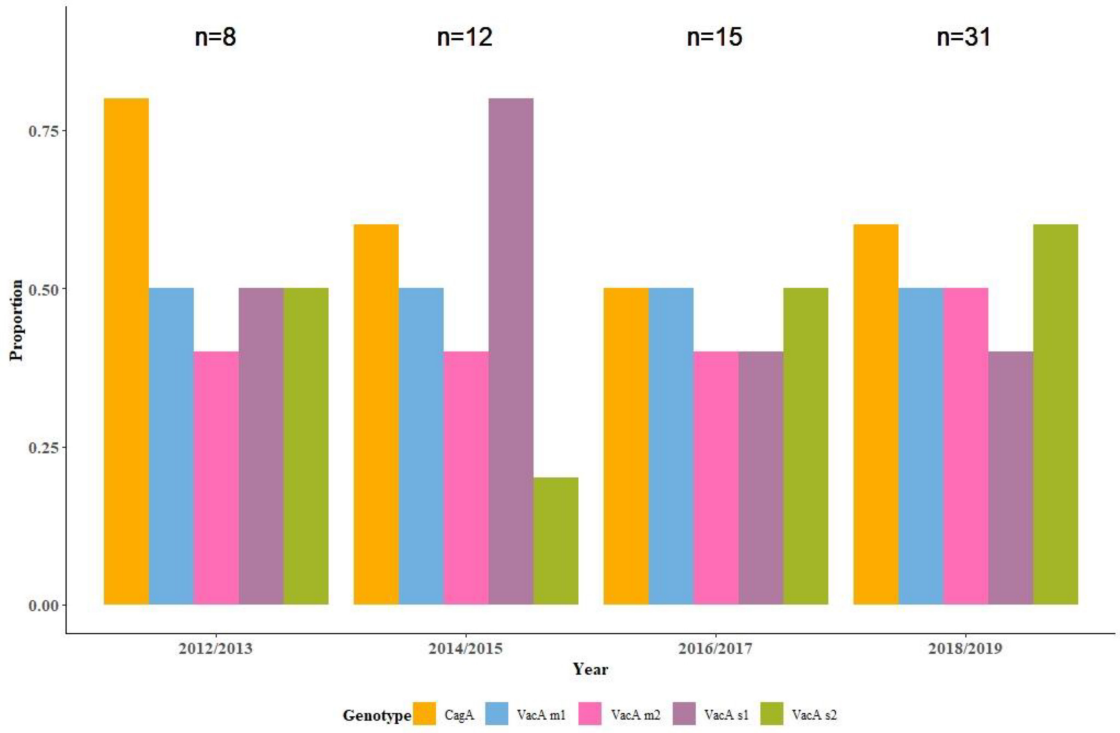

FIGURE 2
Trends in the distribution of *Helicobacter pylori* genotypes (*CagA*, *VacA s1*, *VacA s2*, *VacA m1*, and *VacA m2*) from 2012 to 2019.

antral carcinomas, it is possible that the low yield in this study, was due to the few numbers of antral biopsies. Other reasons could be low efficiency for recovery of bacterial DNA from FFPE, and also the "hit-and-run mechanism," in which the pro-oncogenic actions of *H. pylori* virulence factors are taken over by a series of genetic alterations occurring in cancer-predisposed cells, during long-standing infection (16).

Overall, majority of the cases were poorly differentiated, and further, 60.9% of those positive for *H. pylori* exhibited poor differentiation. This finding was consistent with the concept that malignancies are generally diagnosed at a higher grade in LMIC; and this can be attributed to the absence of screening methods and barriers within the local health system, resulting in late diagnoses.

VacA is considered to be universal to all strains of *H. pylori*; and in brief, it works by binding to the surface of cells and inducing apoptosis, as well as inhibiting the proliferation and immune response of T lymphocytes (17). The "*s*" region corresponds to sequence differences within the terminal signal peptide, and the terminal end of the secreted toxin. The *s2* genotype, in comparison to *s1*, has an impaired ability to form channels in lipid bilayers, and so this allele generally has a reduced capacity to form vacuoles in mammalian cells (18). Therefore, the *s1* genotype is associated with increased severity of disease, compared to *s2*. The "*m*" region of diversity has variable vacuolating activity, which is largely dependent on the type of cell (18). Both alleles, however, have the capacity for vacuolating activity. In agreement with previous studies (9), the current study demonstrated that in comparison with the benign controls, *H. pylori* in the gastric adenocarcinoma cases were more likely *VacA m1* and *VacA s1* genotypes, (OR 1.59 and 2.90, respectively). As such, patients infected by *H. pylori* encoding for the virulent allelic combination *VacA s1m1*, although with wide CI (0.83–8.65), were almost three times more likely to be associated with cancer, than those infected by *H. pylori* of other genotypes. Conversely, the allelic combination of *VacA s2m2*, was almost four times less likely to be associated with cancer. In disagreement with the systematic review and meta-analysis by Pormohammad et al. however, *CagA* genotype in the current study was not associated with a higher risk of gastric cancer development, with an OR of less than one. One of the reasons for this finding, could be that there is low prevalence of the *CagPAI* region, known to enhance its virulence (19), in Kenyan strains of *H. pylori*.

The current study, focusing on *H. pylori* and gastric cancer revealed a divergent risk categorization for *H. pylori* genotypes: *VacA s1m1* with increased risk, and *VacA s2m2* with reduced risk of gastric cancer. Furthermore, over the 8-year study period (2012–2019), there has been an overall increase of *H. pylori* and in the expression of all its genotypes. The genotypes with the greatest year-to-year variation were *VacA s1* and *VacA s2*, (%CV of 41 and 42, respectively), in contrast with, *VacA m1* and *VacA m2*, which exhibited minimal variation (%CV of 4 and 11, respectively). The *CagA* genotype also showed marked variation (%CV 19) over the study duration. These findings would suggest that the evaluation of *VacA s1m1* and other genotypes, as opposed to the mere presence of *H. pylori*, can be used, not only to stratify patients at a higher risk of gastric malignancy, but also for epidemiologic studies.

The study utilized formalin fixed and paraffin embedded tissue blocks as both case and control samples; and despite this method allowing for the preservation of tissue architecture, which in turn allowed for acceptable histologic analyses, formalin is known to cause crosslinking of proteins and nucleic acids, as well as random

breakages in the nucleotide sequences. This in turn could have resulted in false negative results in the molecular analyses of *H. pylori* and its genotypes, as well as failure to detect the expression of some genotypes (see Table 3).

In conclusion, the findings of this study thus put forward, that further assessment of the specific genes encoded by *H. pylori* isolates, in chronically infected persons, can aid in stratifying those at increased risk of development of gastric adenocarcinoma. The low prevalence of intestinal metaplasia and atrophic gastritis also highlights the need for further studies into host *H. pylori* interaction and gastric carcinogenesis in African populations.

Data availability statement

The original contributions presented in this study are included in this article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

PN and AN conceived and designed the study, collected, compiled and analyzed the data, and wrote the final manuscript. ZM and GR participated in the conception and design of the study, providing critical feedback that helped shape the research. YY and ET provided support in the form of training and materials required for the genotype analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1119513/full#supplementary-material

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Immunophenotypic expression profile of multiple myeloma cases at a tertiary hospital in Nairobi Kenya

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Introduction: Multiple myeloma (MM) is a plasma cell neoplasm that constitutes 10–15% of all hematopoietic neoplasms. Kenya is placed among the top five African countries for MM incidence and MM-related mortality. Prior studies have suggested that the aberrant expression of Cyclin D1, CD56, CD117 and Ki-67 on neoplastic plasma cells is useful in disease prognostication. The prevalence and significance of expression of these markers in a cohort of MM cases in Kenya has not been studied previously.

Methods: A retrospective cross-sectional study was carried out at the Aga Khan University Hospital, Nairobi. The study population included 83 MM cases with available trephine blocks archived between 1st of January 2009 and 31st of March 2020. Immunohistochemical expression of Cyclin D1, CD56, CD117, and Ki-67 was analyzed and scored. The biomarkers were described using frequencies based on the positive and negative results. Fisher's exact test was used to determine the association between the immunophenotypic markers and categorical variables.

Results: Of the 83 selected cases, expression of Cyclin D1, CD56, CD117 and Ki-67 was identified in 28.9, 34.9, 7.2, and 50.6%, respectively. Cyclin D1 positivity was significantly associated with hypercalcemia. Absence of CD117 expression was noted to be associated with adverse risk parameters including an IgA isotype or light chain disease, International Staging System (ISS) stage III disease, abnormal baseline serum free light chains (sFLC) and a high plasma cell burden.

Conclusion: Cyclin D1 expression was congruent with previously reported studies. The frequency of CD56 and CD117 expression was lower than previously reported. This may be due to differences in disease biology between the study populations. Approximately half of cases were Ki-67 positive. Our data showed limited associations between the expression of studied markers and clinicopathologic variables. However, this could be attributed to the small study sample size. We would recommend further characterization of the disease in a larger prospective study with the inclusion of survival outcomes and cytogenetic studies.

KEYWORDS

Multiple Myeloma, immunophenotype, CD56, CD117, Cyclin D1, Ki-67, Kenya

Introduction

MM is a plasma cell disorder characterized by multifocal proliferation of neoplastic plasma cells in the bone marrow and is associated with end organ damage related to the disease. Patients may present with bone pain, fractures, hypercalcemia, osteolytic lesions, anemia, recurrent infections, renal insufficiency or a combination thereof (1).

MM constitutes approximately 10–15% of all hematopoietic neoplasms (1). Globally, there were approximately 176,404 new cases and 117,077 deaths attributable to MM in 2020 (2). The incidence of MM has increased uniformly from the 1990s with the highest rise noted in low and middle-income countries. Cowan et al. have reported a low incidence of the disease in Africa (3). However, this likely reflects a paucity of data available due to the absence of high-quality cancer registries as well as diagnostic limitations. According to GLOBOCAN 2020 age-standardized rate (ASR) estimates, Kenya ranked among the top five African countries for MM incidence accounting for 1.7% of new cancer cases and 2.2% of cancer deaths annually (2).

MM is a heterogeneous disease associated with varied outcomes in patients ranging from indolent to extremely aggressive disease. Disparities are driven primarily by differences in underlying disease biology, host factors and therapeutic regimens used. This heterogeneity has been observed among different racial groups. The incidence of MM and Monoclonal Gammopathy of Undetermined Significance (MGUS) is two-to three-fold higher with an earlier age of onset of the disease in those of African descent compared to Caucasians (4–6). United States-based population studies have also indicated a greater overall survival (OS) and disease specific survival (DSS) in patients of African descent when compared to European Americans, suggesting more indolent disease (4, 7). Among other racial groups, no significant differences in survival outcomes have been observed in individuals below 75 years (8).

Some of the most important markers of disease biology include cytogenetic abnormalities, bone marrow plasma cell immunophenotype and plasma cell proliferative rate (9). Studies have suggested a relationship between the aberrant antigenic expression of CD56, CD117, Cyclin D1 and Ki-67 on neoplastic plasma cells and disease prognostication (10). Their assessment may serve as an adjunct to existing risk stratification systems giving further prognostic information especially in those with low or standard risk disease (11). These markers can be assessed by immunohistochemistry (IHC) which is a cost-effective, simple, accessible, and reproducible tool that is routinely used in the diagnosis of MM.

Cyclin D1 (B Cell Lymphoma 1, BCL-1) is a cell cycle regulator aberrantly expressed in approximately 25–50% of MM cases (12). Dysregulation of cyclin D1 has been implicated in the pathogenesis of MM (13). It therefore serves as a potential therapeutic target (14). Cyclin D1 expression on plasma cells has been associated with t (11;14) that juxtaposes the Cyclin D1 gene to IgH enhancer elements resulting in its upregulation (15–17). t (11;14) is the most common IgH translocation identified in MM cases particularly among individuals of African Ancestry (18). Baughn et al. consequently suggested it may be one of the drivers of the racial disparity observed in MM (18). t (11;14) is generally considered to be a marker of standard risk disease as described in the Revised-International Staging System (R-ISS) (19). Studies have however had conflicting results on the prognostic significance of cyclin D1 surface expression (20–23).

CD56 (Neural Cell Adhesion Molecule, NCAM) is a membrane glycoprotein which is expressed in 70–80% of MM cases (1). It is suggested to be involved in the homing of MM cells to the bone marrow matrix. Lack of CD56 expression is associated with higher levels of bone marrow infiltration and peripheral blood involvement with an associated adverse prognosis (24–26). Conversely, CD56 expression has been associated with an increased treatment response to bortezomib and independently linked to longer overall survival in patients (11, 26–28).

CD117 (c-kit/stem cell factor receptor) is a transmembrane hematopoietic growth factor receptor with tyrosine kinase activity. It is overexpressed in approximately 20–30% of patients with MM (1). CD117 negative MM has been demonstrated to have a poor prognosis with shorter progression free survival (PFS) and overall survival (OS) (29–31). Bataille et al. found the poorer survival conferred by the absence of this marker to be independent of the treatment received (29).

Plasma cell labeling index has traditionally been used to assess the proliferative activity of myeloma cells. However due to technical demands, its use has been limited. Several studies have therefore examined the expression of Ki-67 in MM as a marker of cellular proliferation and prognosis. A high Ki-67 index is a poor prognostic marker correlating with a shorter OS (32, 33).

There is a paucity of data on MM from Sub-Saharan Africa. Prior studies conducted in Kenya have reported on clinicopathologic parameters and patient survival (5, 6). However, the plasma cell immunophenotype in a cohort of patients with MM has not been studied previously. This study was undertaken to describe the immunophenotypic expression profile of CD 56, CD 117, Cyclin D1 and Ki-67 in trephine biopsies of patients diagnosed with MM at the Aga Khan University Hospital, Nairobi and to evaluate their association to clinicopathologic findings and risk stratification parameters at diagnosis.

Materials and methods

This was a retrospective cross-sectional laboratory-based study, which was undertaken at the Department of Pathology at the Aga Khan University Hospital (AKUH) Nairobi, a private nonprofit institution. The AKUH laboratory receives specimens from institutions across Kenya. The study was approved by the Aga Khan University Research Ethics Committee {Ref: 2019/IERC-97 (v3)} and was in line with the declaration of Helsinki and REMARK guidelines (34, 35).

Sample collection

A total of 151 cases of MM were identified between 1st of January 2009 and 31st of March 2020. Of these, 83 cases were selected based on the following criteria:

a) Patients diagnosed with MM according to the International Myeloma Working Group criteria (IMWG) (36).
b) Treatment naïve, relapsed, or refractory cases of MM

c) Availability of sufficient tumor representative areas in the trephine blocks.

A census approach was used to collect data on all the trephine biopsies of MM cases. The sampling protocol is illustrated in Supplementary Figure S1.

Data collection

Patients' medical records were reviewed to obtain clinical information. The data collected included age at diagnosis, gender, hematologic and biochemical parameters including those related to disease staging (International Staging System), patient disease status (treatment naïve/relapsed or refractory) and treatment regimens used.

Immunohistochemical expression

The formalin-fixed, paraffin-embedded (FFPE) trephine specimens were coded using serial numbers. CD 138 slides (to assess for plasma cells) were retrieved from the archives along with the trephine blocks. For cases where these were unavailable, new slides were prepared.

Serial sections of approximately 4 µm were collected from the FFPE trephine blocks onto Poly-L-lysine coated slides. The sections were de-waxed. Antigen retrieval was done using the Dako target-antigen retrieval solution, pH 9, which utilizes the principle of heat-induced epitope retrieval. Immunohistochemical staining for CD138, Cyclin D1, CD56, CD117, and Ki-67 was then performed using the Dako Autostainer Link 48 (Agilent Technologies, Dako Denmark A/S) according to the manufacturer's recommendations. Details of the antibodies used are summarized in Supplementary Table S1. Appendiceal and tonsillar tissue sections were used as controls.

Plasma cell burden was estimated using CD138 expression and scored in 10% increments. The relative percentage of positive cells for each IHC marker was estimated in relation to plasma cell staining (37). A minimum of 500 neoplastic cells were evaluated. Each case was scored as positive for a marker using the scoring criteria outlined in Table 1 below regardless of the staining intensity.

The cut-offs used were based on protocols used in previous studies (21–23, 38, 39). Appropriate localization for the specific staining was also considered. The marker staining was evaluated alongside appropriate on-slide controls.

All the slides were examined by the principal investigator and two hematopathologists who were blinded for the patient's medical history. Discordant results were subjected to consensus decision.

Statistical analysis

Collected data was entered in Microsoft Excel spreadsheets and analyzed using EXCEL and IBM Statistical Package for Social Sciences SPSS version 20 (IBM Corp., Armonk, N.Y., United States) software. Continuous data was expressed as means and medians. Patient and laboratory characteristics were recorded as categorical variables and summarized using frequencies and percentages. The IHC markers were described using frequencies with corresponding percentages and corresponding 95% confidence intervals. Fisher's exact test was used to determine the association between the expression of immunophenotypic markers and categorical variables. Kruskal Wallis test was used for continuous data. A p value of <0.05 was considered significant.

Results

Sample characteristics

A total of 83 cases (36 females and 47 males) were included in the study. The median age was 61 years with almost half of the patients being between 50 and 64 years of age at the time of diagnosis. Only 15 (18%) patients were below 50 years and 11 (13.3%) were more than 75 years of age. The demographic and clinicopathologic data are listed in Supplementary Table S2. Patient laboratory parameters are summarized in Table 2. The clinicopathologic data were unavailable in some cases.

The most common immunoglobulin isotype was IgG (55.8%) followed by IgA (25.6%) with kappa being the predominant involved light chain (60.4%). Seven patients (16.3%) had light chain disease.

Notably, more than half of patients (56.8%) had ISS stage III disease. Elevated levels of beta-2 microglobulin (B2M) were identified in 64.1% of cases with a mean value of 7.2 mg/l.

For those whose treatment data was available, majority of the patients had been started on bortezomib and lenalidomide-based chemotherapy regimens. The most common regimen was bortezomib/

TABLE 1 Scoring criteria.

Antigen	Localization	Scoring criteria	
		Negative	Positive
Cyclin D1	Cytoplasmic	<10%	>10% (22, 38)
CD56	Cytoplasmic	<10%	>10% (21)
CD117	Cytoplasmic or membranous	<10%	>10% (23)
Ki-67	Nuclear	<10%	>10%[a](38, 39)

[a]A Ki-67 score of >10% was further classified as intermediate (10–20% Ki-67 positive plasma cells) or high (>20% Ki-67 positive plasma cells) (38, 39).

TABLE 2 Patient laboratory parameters.

Laboratory parameter	No.	Mean	Median	Range
Hemoglobin (g/L)	71	9.9	10.1	4.1–16.4
Creatinine(µmol/L)	50	187.5	108.4	22–802
Urea (mmol/L)	49	9.2	5.7	1.8–33.5
Calcium (mmol/L)	44	2.5	2.4	1.86–4.5
LDH (U/L)	33	277.9	213	97–1,317
Total protein (g/L)	51	89.8	82.9	47.3–147.9
Albumin (g/L)	50	34.2	34.6	18.2–48
β-2microglobulin (mg/L)	39	7.2	5.3	1.18–36.1

lenalidomide/dexamethasone (VRd) triple therapy, which was administered to 47.6% of patients. The findings are summarized in Figure 1.

The study cohort included 67 (80.7%) treatment naïve and 16 (19.3%) refractory or relapsed (RR) cases. The RR group was more likely to have an IgA isotype (60% verus 21.1%) or light chain disease (40% versus 13.2%) compared to treatment naïve cases as shown in Supplementary Table S3.

Frequency of antigen expression

The IHC expression profile of Cyclin D1, CD56, CD117 and Ki-67 is detailed in Table 3 below.

Of the Ki-67 positive cases, 37 (44.6% of total cases) had a high Ki-67 expression and 6 cases (7.2%) were classified as intermediate.

Representative photomicrographs of the IHC staining are shown in Figure 2.

Comparison of antigen expression in treatment naïve versus relapsed or refractory (RR) cases

There was a higher frequency of Ki-67 positivity in the RR compared to the treatment naïve group (62.5% versus 49.3%). All RR cases were also noted to be CD117 negative (Table 4).

Of the positive Ki-67 RR cases, 44.8% had a high level of Ki-67 expression and 4.5% had an intermediate Ki-67.

A diagnostic trephine was available for four RR cases. All four were negative for Ki-67 at diagnosis and only one case developed Ki-67 positivity at relapse. None of these cases had a change in the expression of Cyclin D1, CD56 or CD117 from diagnosis.

Association between the immunohistochemical markers and clinicopathologic variables

Clinicopathologic parameters including anemia, renal insufficiency, hypercalcemia, elevated lactate dehydrogenase (LDH), elevated B2M, abnormal serum free light chain ratio (sFLC), ISS stage, immunoglobulin isotype and the involved light chain were recorded and analyzed for association with antigen marker expression.

Hypercalcemia was significantly associated with the expression of cyclin D1 ($p = 0.042$). No other significant associations were observed (Supplementary Table S4).

There were no significant associations between CD56 expression and any of the clinicopathologic variables recorded (Supplementary Table S5).

All cases with an IgA isotype or light chain disease lacked CD117 expression on trephine biopsy as did a majority (85.7%) of patients with ISS stage III disease. Cases that lacked CD117 expression were also more likely to have an abnormal sFLC ratio (83% of cases) and a high plasma cell burden of >50% (63.9% of cases). However, associations between CD117 expression and clinicopathologic variables did not reach statistical significance (Supplementary Table S6).

There was no association between Ki-67 and any of the clinicopathologic parameters recorded (Supplementary Table S7).

Discussion

In this study, we describe the immunophenotypic expression profile of MM cases with reference to Cyclin D1, CD 56, CD117 and Ki-67 expression. We also sought to evaluate the association of the expression of these markers with clinicopathologic findings and risk stratification parameters. To the best of our knowledge, this is the first study to evaluate these IHC markers in a cohort of MM cases in Sub-Saharan Africa.

Our findings from the demographic variables in this study were similar to those from previous MM studies carried out in Sub-Saharan Africa that have reported a male predominance and median age at presentation of between 53 to 62 years (5, 6, 40–42). This is a younger age at diagnosis than described in studies conducted in predominantly Caucasian populations such as that by Kyle et al. and Kristinsson et al. who reported a median age of 66 and 70 years, respectively, (43, 44). Other studies have demonstrated that individuals of African descent have a younger age of disease onset as compared to Caucasians (4, 8).

The International Myeloma Working Group (IMWG) diagnostic criteria incorporates the presence of renal insufficiency, anemia and hypercalcemia, which signify end-organ damage attributable to the

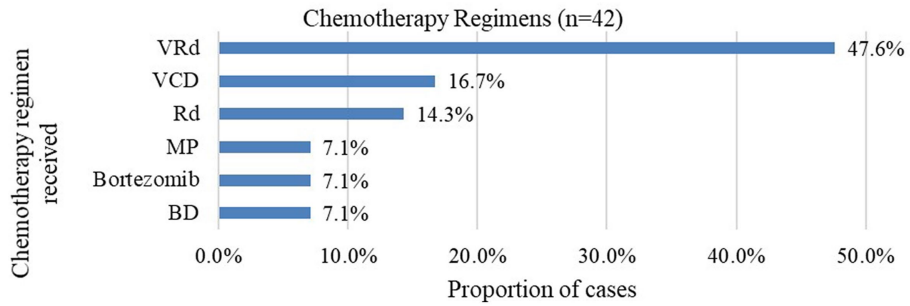

FIGURE 1
Proportion of chemotherapy regimens received at baseline. VRd bortezomib/lenalidomide/dexamethasone; VCD bortezomib/cyclophosphamide/dexamethasone; Rd. lenalidomide/dexamethasone; MP melphalan/prednisone; BD bortezomib/dexamethasone.

TABLE 3 Immunophenotypic expression frequency (%) of biomarkers.

Markers		*n*	%	95% CIs
Cyclin D1	Positive	24	28.9%	[0.191–0.387]
	Negative	59	71.1%	[0.613–0.809]
CD56	Positive	29	34.9%	[0.246–0.452]
	Negative	54	65.1%	[0.548–0.754]
CD117	Positive	6	7.2%	[0.017–0.128]
	Negative	77	92.8%	[0.872–0.984]
Ki67	Positive	43	51.8%	[0.398–0.614]
	Negative	40	48.2%	[0.374–0.590]

disease. Anemia was present in almost half (47.9%) of cases in this study similar to the frequency described in previous Kenyan studies (5, 6, 42). This is however much lower than reports from studies conducted in other countries in Sub-Saharan Africa and the USA that recorded frequencies of between 71 to 77% (40, 43, 45). Renal failure was present in 40% of cases comparable to earlier findings by Kiraka et al. at the same study center (5). Other studies have reported varying frequencies ranging from 13 to 33%. The frequency of hypercalcemia (18.2%) was comparable to that reported by Kyle et al. and Otieno-Abinya et al. (13 and 19% respectively) but lower than described in other Kenyan studies that ranged from 34 to 54% (5, 6). Data on creatinine and calcium levels were available in just over half of the cases therefore our findings may not be fully representative of the study population.

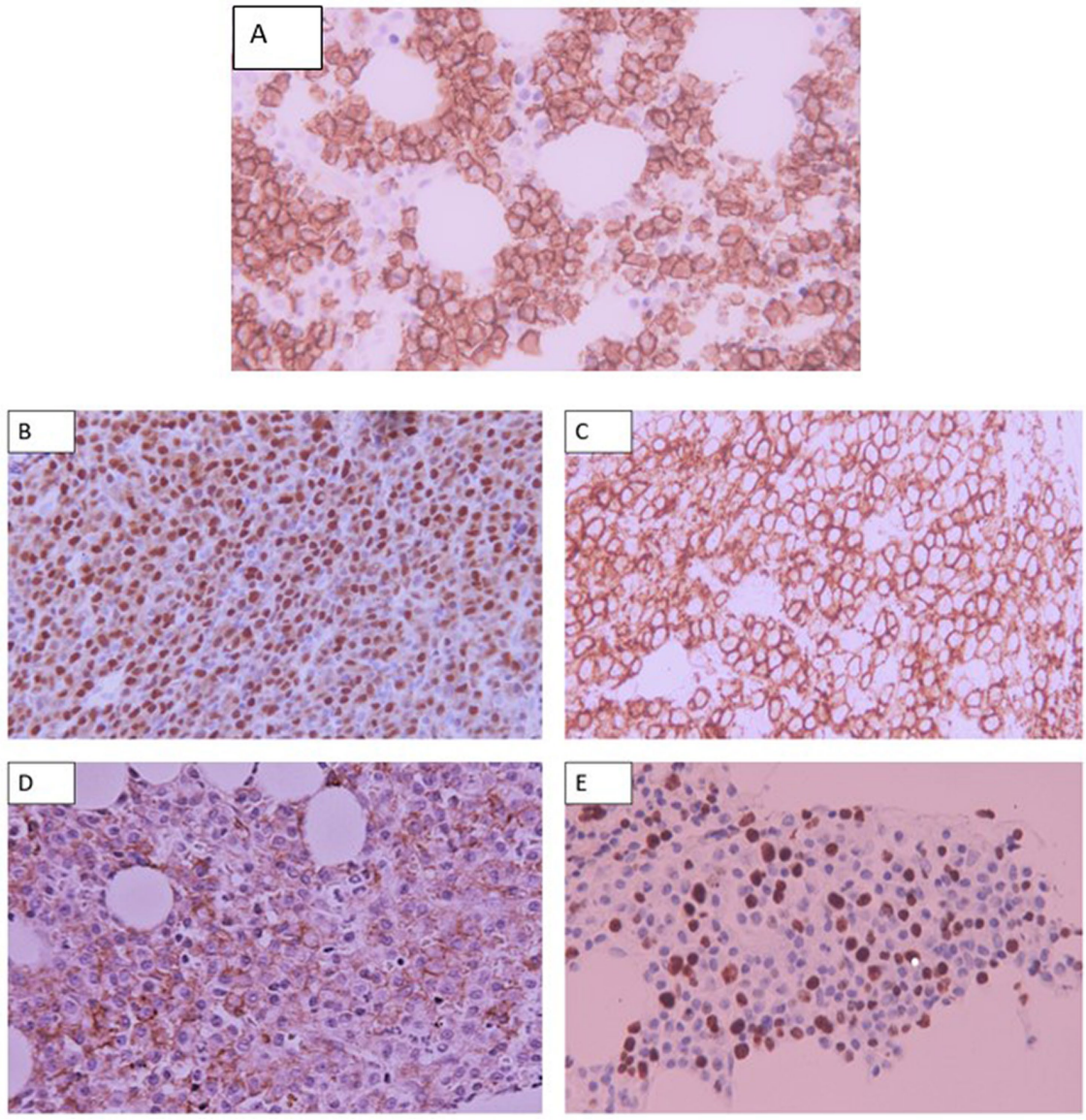

FIGURE 2
Representative photomicrographs of: **(A)** Membranous staining of CD138 of plasma cells. **(B)**Nuclear staining of Cyclin D1 on MM cells. **(C)** Membranous staining of CD56 by MM cells. **(D)** Membranous staining of CD117 on MM cells. **(E)** Nuclear staining of Ki-67 by MM cells. Staining of other hematopoietic elements is also appreciated. Staining was analyzed using Olympus microscope CX23 at a magnification of X400 and images were acquired using a Canon camera.

TABLE 4 Comparison of the immunophenotypic expression profile in treatment naïve versus refractory/relapsed cases.

		Naïve (*n*=67)		Refractory and Relapsed (*n*=16)	
Cyclin D1	Positive	19	28.4%	5	31.3%
	Negative	48	71.6%	11	68.8%
CD56	Positive	24	35.8%	5	31.3%
	Negative	43	64.2%	11	68.8%
CD117	Positive	6	9.0%	0	0.0%
	Negative	61	91.0%	16	100.0%
Ki67	Positive	33	49.3%	10	62.5%
	Negative	34	50.7%	6	37.5%

Hypoalbuminemia, elevated B2M and LDH levels predict for a high plasma cell burden and an adverse prognostic risk in MM. Albumin and B2M levels form the criteria for ISS staging while LDH has been incorporated into the R-ISS staging system. Elevated LDH levels and hypoalbuminemia were observed 48.5 and 52% of cases, respectively. This is much higher than described in a study by Kyle et al. that reported frequencies of 10 and 15%, respectively, in a cohort of MM cases in the USA (43). The findings are however comparable to those described in other studies conducted in Kenya, Ghana and Uganda (6, 40, 46). 64.1% of cases also notably presented with an elevated B2M which is comparable to a frequency of 75% reported by Kyle et al. (43). Additionally, more than half of cases (56.8%) in this study had ISS stage III disease similar to the finding of 51.3% by Acquah et al. in Ghana but higher than the estimated global frequency of 33.6% (40, 47). These findings suggest that approximately half or more of MM cases present with advanced disease in our study population, as well as in some of the other Sub-Saharan countries described. This may be attributable to delays in presentation and referral as well as limited access to diagnostic services and treatment in low-and-middle income-countries.

In keeping with previous studies, IgG was the most common immunoglobulin isotype at 55.8% with the involved light chain being predominantly kappa at 60.4% (48). 16% of patients had light chain disease, similar to earlier studies (5, 43). Refractory cases were more likely to have an IgA isotype or light chain disease which is an expected finding as both isotypes are associated with an unfavorable outcome (48). It has also been reported that up to 96% of MM cases have an abnormal sFLC ratio at diagnosis (49). This is congruent with the frequency of 92.9% found in this study.

Cyclin D1 expression was demonstrated in 28.9% of cases, in keeping with the previously reported frequency of between 25–50% (16, 17, 20, 22, 23, 38). The expression of the antigen is upregulated by t (11;14) and the immunohistochemical expression has been shown to correlate well with the presence of the cytogenetic anomaly (16). Although it may not be routinely tested, the assessment of this marker would be useful in the small cell variant of MM as these cases have previously been demonstrated to have strong Cyclin D1 positivity and to be associated with t (11;14) (1, 17). This can be particularly useful in resource-limited settings where cytogenetic studies for t (11;14) may not be readily accessible. t (11;14)is the most common IgH translocation in MM particularly among persons of African descent (18). For this reason, Cyclin D1 expression may have been hypothesized to be higher than was observed given that the study was conducted in a predominantly African population. The presence of this translocation in Cyclin D1 expressing cases would however be better confirmed by cytogenetic studies (16, 17).

t (11;14) that upregulates Cyclin D1 expression is considered a standard risk cytogenetic anomaly in MM according to the R-ISS staging system (19). Studies have however had conflicting results on the prognostic significance of cyclin D1 surface expression (20–23). There was no significant difference in Cyclin D1 expression between treatment naïve and RR cases similar to prior studies by Pruneri et al. and Kelley et al. (16, 20). Athanisou et al. documented an association between marker expression and plasma cell burden (22). In this study, Cyclin D1 expression was significantly found to be associated with hypercalcemia ($p = 0.042$) in contrast to findings by Markovic et al. (38).This finding cannot be viewed in isolation however as there were no other significant associations noted. A number of previous studies similar to this study did not demonstrate any association between Cyclin D1 expression and the other adverse risk variables (16, 17, 38). As cyclin D1 expression may signify standard risk disease, the limited association between this antigen and the included adverse risk clinicopathologic variables is expected.

An unanticipated low expression of CD56 was observed in the present study at 34.9% in contrast to the previously reported frequency of 70–80% (11, 26, 28, 50). The CD117 expression frequency of 6% was also much lower than the range of 24–40% reported in prior studies (11, 16, 28). Of note, the studies showing higher expression of CD56 and CD117 had been conducted in predominantly Caucasian populations. Only two of these studies to the best of our knowledge had been carried out in Africa -Meddour et al. in Algeria and Khallaf et al. in Egypt (51, 52). None had been conducted in Sub-Saharan Africa. The unexpected findings may represent a difference in disease biology between the studied populations. The findings from our study should be confirmed with a larger cohort.

Lack of CD56 expression has been associated with a number of adverse clinicopathologic factors including higher B2M levels and higher incidence of renal insufficiency and therefore thought to represent more aggressive disease (26, 50). However, there have been few exceptions to these findings such as the study by Khallaf et al. which similar to the present study did not demonstrate this (52). The significance of the low CD56 expression in our population remains uncertain and should be further assessed.

CD 117 expression in MM has been associated with more indolent disease which is supported by evidence of its frequent expression in MGUS (29). Conversely, CD 117 negative cases have been associated with adverse risk clinicopathologic parameters and shorter PFS and OS (29–31). Although no significant associations were identified between CD117 expression and the clinicopathologic variables, cases that lacked CD117 expression were more likely to have an IgA isotype or light chain disease, advanced ISS stage and a high plasma cell burden. Mateo et al. similarly reported increased bone marrow infiltration and advanced ISS in CD117 negative MM cases (30). However, unlike Mateo et al. and Ceran et al., we did not find any associations with other adverse variables including anemia, renal impairment and elevated B2M or LDH levels (30, 31). In the

comparison of CD117 expression in the trephine biopsies of treatment naïve and RR cases, all RR cases were CD117 negative. Bataille et al. similarly reported a lower rate of CD117 expression in relapsed disease compared to newly diagnosed cases (8% versus 33%) (29). The study findings seem to support the adverse risk associated with absence of CD117 expression in MM, which a larger sample may have brought out more clearly.

Overall, approximately half (50.6%) of cases had a positive Ki-67 with 44.6% having a high level of expression. A high Ki-67 index is a poor prognostic marker in MM correlating with a shorter OS (32, 33). It has also been linked to angiogenic activity which correlates with disease progression and tumor burden in myeloma (53). It would therefore seem that almost half of the study cases had poor risk disease. The finding that 56.8% of cases in this study had ISS stage III disease would seem to support this. The apparent high prevalence of advanced disease may be due to delays in presentation, diagnosis, or referral. As has been suggested by previous authors, Ki-67 may be offered at diagnosis to identify high risk cases (54). It can serve as an adjunct to existing risk stratification systems providing further prognostic information. Ki-67 expression was noted in 49.3% of the treatment naïve cases. This was slightly higher than the findings by Himani et al. who reported a frequency of 33.9% in their study population (54).The higher Ki-67 positivity observed in RR cases compared to newly diagnosed cases (62.5% versus 49.3%) in this study was also in keeping with the poor risk associated with the marker.

In analyzing the association between Ki-67 expression and the clinicopathologic variables, none was demonstrated as statistically significant. This is in contrast to a study by Alexandrakis et al. that demonstrated an association between Ki-67 expression and markers that predict for a high plasma cell burden including elevated LDH and B2M levels (55). However, Himani et al. similar to the present study did not find any association between antigen expression and serum calcium, creatinine or B2M levels (54). The heterogeneity of findings between this study and others describing the frequency of the IHC markers and their association with clinicopathologic findings may be due to differences in sample size, study design and methodology.

This study had some limitations including a small sample size and incomplete clinical data including information on survival outcomes that would have enriched the study. Some cases were received from external centers for evaluation while others were lost to follow-up, which contributed to the incomplete data. Additionally, patient financial constraints meant that some routine laboratory investigations could not be carried out at presentation. Furthermore, although inclusion of cytogenetic studies would have provided powerful prognostic information, they are largely unaffordable and inaccessible to the general Kenyan population and are therefore not routinely performed for MM cases at the study center.

Conclusion

This study is one among few in Africa to describe the expression of Cyclin D1, CD 56, CD 117, and Ki-67 and their association with clinicopathologic findings and risk stratification parameters in a cohort of MM cases. From our findings, the utility of the studied IHC markers in MM prognostication remains uncertain. However, Ki-67 expression was high in this study cohort and due to its correlation with aggressive disease, it may be assessed at diagnosis as an adjunct to existing risk stratification systems to provide further prognostic information. Cyclin D1 expression was similar to what has previously been reported. The assessment of this marker would be useful in the small cell variant of MM, as these cases have previously been demonstrated to have strong Cyclin D1 positivity and to be associated with t (11; 14). CD56 and CD117 expression was low in this study cohort compared to prior studies. This may be due to differences in disease biology between the study populations. We recommend further prospective studies to validate our findings and further characterize the disease in a larger cohort with the inclusion of survival outcomes and cytogenetic studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Aga Khan University Research Ethics Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

IM: participated in the conception and design of the study, data collection and analysis and writing of the final manuscript. EK and AM: participated in the conception and design of the study, data collection and critical review of the manuscript. EL and SR: participated in the conception and design of the study and critical review of the manuscript. RM and ZM: participated in the design of the study, data collection and provision of critical feedback that helped shape the research. All authors listed have made a substantial and direct contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1177775/full#supplementary-material

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The diagnostic accuracy of an initial point-of-care lactate at the emergency department as a predictor of in-hospital mortality among adult patients with sepsis and septic shock

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Background: In patients with sepsis, elevated lactate has been shown to be a strong predictor of in-hospital mortality. However, the optimal cutoff for rapidly stratifying patients presenting to the emergency department at risk for increased in-hospital mortality has not been well defined. This study aimed to establish the optimal point-of-care (POC) lactate cutoff that best predicted in-hospital mortality in adult patients presenting to the emergency department.

Methods: This was a retrospective study. All adult patients who presented to the emergency department at the Aga Khan University Hospital, Nairobi, between 1 January 2018 and 31 August 2020 with suspected sepsis or septic shock and were admitted to the hospital were included in the study. Initial POC lactate results (GEM 3500® blood gas analyzer) and demographic and outcome data were collected. A receiver operating characteristic (ROC) curve for initial POC lactate values was plotted to determine the area under the curve (AUC). An optimal initial lactate cutoff was then determined using the Youden Index. Kaplan–Meier curves were used to determine the hazard ratio (HR) for the identified lactate cutoff.

Results: A total of 123 patients were included in the study. They had a median age of 61 years [interquartile range (IQR) 41.0–77.0]. Initial lactate independently predicted in-hospital mortality [adjusted odds ratio (OR) 1.41 95% confidence interval (CI 1.06, 1.87) $p = 0.018$]. Initial lactate was found to have an area under the curve (AUC) of 0.752 (95% CI, 0.643 to 0.86). Additionally, a cutoff of 3.5 mmol/L was found to best predict in-hospital mortality (sensitivity 66.7%, specificity 71.4%, PPV 70%, NPV 68.2%). Mortality was 42.1% (16/38) in patients with an initial lactate of $\geq$ 3.5 mmol/L and 12.7% (8/63) in patients with an initial lactate of $<$3.5 mmol/L (HR, 3.388; 95% CI, 1.432–8.018; $p < 0.005$).

Discussion: An initial POC lactate of $\geq$ 3.5 mmol/L best predicted in-hospital mortality in patients presenting with suspected sepsis and septic shock to the emergency department. A review of the sepsis and septic shock protocols will help in the early identification and management of these patients to reduce their in-hospital mortality.

KEYWORDS

sepsis, septic shock, lactate, hospital mortality, outcomes, point of care systems, emergency care

1. Introduction

The global epidemiological burden of sepsis is difficult to ascertain in part due to challenges in clinical definitions and heterogeneity in sepsis coding and reporting in clinical databases (1). There is also a paucity of data due to critical shortages in healthcare workers and a lack of access to laboratory facilities, especially in sub-Saharan Africa (SSA) (2). In Kenya, there is heterogeneity in case definitions of sepsis with prevalence ranging from 10% in adults with suspected sepsis in critical care units to 23.9% in neonatal sepsis (3, 4).

Sepsis is a clinical syndrome rather than a specific illness. This confers a diagnostic challenge given the variability in clinical signs and symptoms as well as the lack of an agreed-upon standard diagnostic test (5). One of the biomarkers featured in the sepsis guidelines is the measurement of lactate (6). Lactate levels have been long associated with tissue hypoperfusion, thus its incorporation into the clinical definitions of formerly severe sepsis and septic shock. There is now evidence that shows multifactorial causes of sepsis-associated hyperlactatemia including accelerated aerobic glycolysis, cytopathic hypoxia, direct mitochondrial impairment, and dysfunction of hepatic lactate clearance. In addition, lactate levels have been shown to strongly predict in-hospital mortality in patients with sepsis and septic shock (7–10).

There are several obstacles to the rapid determination of lactate levels in the emergency department in low- and middle-income countries (LMICs). The lack of core laboratory services may play a role in the underutilization of lactate testing in sepsis care (11, 12). In addition, prolonged turnaround time with the use of core laboratory analysis results in further limitations, as the test result must actively be sought out by the treating physician (13). Rapid testing of lactate in the emergency department in line with the sepsis bundles can be achieved with point-of-care (POC) testing, allowing for the implementation of a screening protocol in patients with suspected sepsis. POC testing allows for rapid testing with bedside results allowing for immediate intervention. It also allows for improved accuracy over core laboratory testing encumbered by pre-analytical errors such as prolonged tourniquet time or delayed centrifugation (13).

In the emergency department, lactate can be used as a marker of effective resuscitation, identification of patients with occult hypotension, risk stratification of patients, and as a mortality prediction tool (14). However, there is a lack of consensus on the optimal lactate cutoff that best predicts in-hospital mortality (15). The cutoff of 2 mmol/L is recommended by the Surviving Sepsis Campaign and incorporated into the 1 h sepsis bundle. They however caution that the recommendation is weak based on a low quality of evidence. Additionally, no studies from LMICs including SSA were included (6). There is a heterogeneity in the cutoffs proposed by different studies, using different lactate measurement platforms [central analyzers, point-of-care (POC) blood gas analyzers, handheld POC lactate devices], different patient populations, and different sample types including whole blood (arterial, venous, and capillary) and serum/plasma samples (10, 14, 16–19).

This study aimed at determining a POC blood gas lactate cutoff that best predicted in-hospital mortality in patients with suspected sepsis and septic shock presenting to the emergency department.

2. Methods

2.1. Study design and setting

We conducted a cross-sectional study at the Aga Khan University Hospital, Nairobi (AKUH, N), where medical records for patients admitted with a diagnosis of sepsis or septic shock as per the third international consensus definitions for sepsis and septic shock guidelines (SEPSIS-3) (5) criteria between 1 January 2018 and 31 August 2020 were reviewed retrospectively. Sepsis was defined as the presence of infection with signs of organ dysfunction which were represented by a Sequential Organ Failure Assessment (SOFA) score of two points or greater. Septic shock was defined as a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and a serum lactate level of >2 mmol/L in the absence of hypovolemia (5).

The Aga Khan University Hospital Nairobi Research Ethics Committee approved the study (2020-IERC/142).

2.2. Selection of participants

Patients with an initial POC lactate result from whole blood (arterial and venous) samples measured on the GEM 3500® blood gas analyzer at admission were included. Patients with no outcome data available were excluded. All patient identifiers were removed during the data extraction process.

2.3. Data collection

We screened all admission records during the study period to recruit those who met the inclusion criteria. The following data were extracted using a data collection tool: patient demographic data, initial lactate result at admission from the emergency department obtained from the GEM 3500® blood gas analyzer, an initial SOFA score, the focus of infection, patient comorbidities, the final diagnosis at discharge or death, and the length of hospital stay.

2.4. Data analysis

The data analysis was carried out using IBM Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp., Armonk, N.Y., USA) software.

The study population was described using demographic, clinical, and laboratory characteristics. Descriptive quantitative variables were reported using means (± standard deviation) or medians [interquartile range (IQR)] according to their distribution. The chi-square test or Fischer's exact test compared categorical variables where appropriate.

TABLE 1 Demographics and clinical characteristics.

	All patients ($N = 123$)	Survivors ($N = 95$)	Non-survivors ($N = 28$)
Demographics:			
Age (years) [Median (IQR)]	61.0 [41.0, 77.0]	61.0 [40.0, 75.0]	68.0 [50.0, 78.5]
Gender, *n* (%):			
Male	65 (52.8)	46 (48.4)	19 (67.9)
Female	58 (47.2)	49 (51.6)	9 (32.1)
Co-morbidities [*N* (%)]:			
Diabetes	31 (25.2)	23 (24.2)	8 (28.6)
Hypertension	46 (37.4)	39 (41.4)	7 (25.0)
Renal disease	26 (21.1)	24 (25.3)	2 (7.1)
Malignancy	38 (30.9)	22 (23.2)	16 (57.1)
Neurological disorders	26 (21.1)	19 (20.0)	7 (25.0)
HIV	14 (11.4)	9 (9.5)	5 (17.9)
Pulmonary disease	4 (3.3)	3 (3.2)	1 (3.6)
Liver disease	4 (3.3)	3 (3.2)	1 (3.6)
Focus of infection found [*N* (%)]:			
Yes	94 (76.4)	78 (82.1)	16 (57.1)
No	29 (23.6)	17 (17.9)	12 (42.9)
Site of infection [*N* (%)]:			
Respiratory	35(37.2)	28(35.9)	7(43.8)
Renal	18 (19.1)	17 (21.8)	1 (6.2)
Bloodstream	14 (14.9)	12 (15.4)	2 (12.5)
Abdominal	14 (14.9)	12 (15.4)	2 (12.5)
Skin/soft tissue infections	12 (12.8)	8 (10.3)	4 (25.0)
Central nervous system	1 (1.1)	1 (1.3)	0 (0.0)
SOFA score [Median (IQR)]:	5.0 [3.0,7.0]	4.0 [3.0, 6.0]	7.0 [4.0, 9.5]
Length of hospital stay:			
Length of stay in hospital (days) [Median (IQR)]	7.0 [4.0, 13.0]	7.0 [4.0, 14.0]	8.0 [2.0, 14.0]
Lactate:			
Initial lactate [Median (IQR)]	3.0 [2.0, 5.0]	2.0 [2.0, 4.0]	4.0 [3.0, 8.5]

The association between known risk factors and in-hospital mortality was determined using regression analysis. The selection of the risk factors, including age, gender, SOFA score, malignancy, and renal failure, was based on prior studies performed on patients admitted with sepsis from the emergency department. These variables were found to be associated with increased in-hospital mortality (20, 21).

A receiver operating characteristic (ROC) curve for initial POC lactate values was plotted to determine the area under the curve (AUC). The optimal lactate cutoff that best predicted in-hospital mortality was determined by calculating the Youden Index. Survival analysis was performed using Kaplan–Meier curves to determine the hazard ratio for the identified lactate cutoff. A p-value of <0.05 was considered to be statistically significant.

3. Results

3.1. Demographic and clinical characteristics

A total of 159 patients were admitted to the emergency department with a diagnosis of sepsis or septic shock. Of these, 36 were excluded due to missing lactate values or incomplete outcome data. The final study cohort comprised 123 patients, and their demographic and clinical characteristics are presented in Table 1.

The median age of the patients was 61 years (IQR 41.0–77.0), and more than half (52.8%) of the patients were male. The most common co-morbidity was hypertension in 46 patients (37.4%) followed by malignancy in 38 patients (30.9%). The all-cause mortality rate was 22.8% (95% CI: 15.4–30.2%). Those who died

TABLE 2 Associations between risk factors and in-hospital mortality.

	Univariate analysis		Multivariate analysis	
	Unadjusted OR (95% CI)	*p*-value	Adjusted [a] OR (95% CI)	*p*-value
Age	1.01 (0.99–1.03)	0.340	1.01 (0.98–1.04)	0.523
Gender (male)	2.27 (0.93–5.56)	0.074	1.79 (0.48–6.25)	0.401
SOFA score	1.22 (1.07–1.39)	0.004	1.21 (0.97–1.52)	0.093
Malignancy	4.15 (1.69–10.16)	0.002	4.19 (1.24–14.20)	0.021
Renal disease	0.22 (0.05–1.03)	0.055	0.05 (0.004–0.61)	0.019
Initial lactate	1.41 (1.16–1.71)	<0.001	1.41 (1.06–1.87)	0.018

[a] Adjusted for age, gender, SOFA score, malignancy, and renal disease.

had a longer median length of hospital stay, but this was not statistically significant [8.0 (2.0, 14.0) vs. 7.0 (4.0, 14.0), $p = 0.901$].

The focus of infection was identified in 94 patients (76.4%) with these patients having a better survival compared to the "no focus" group, 82.1 vs. 42.9%, respectively ($p = 0.011$). The most common site of infection was the respiratory system (37.2%) followed by the renal system (19.1%). However, there was no statistically significant difference in survival based on the sites of infection ($p = 0.503$).

Increasing SOFA scores were associated with poorer outcomes with a median SOFA score of 7.0 [4.0, 9.5] in the non-survivors compared to 4.0 [3.0, 6.0] in the survivors ($p = 0.004$).

3.2. Initial POC lactate

Initial lactate values ranged between 0.5 and 15 mmol/L with the median initial lactate at presentation being 3.0 mmol/L (IQR 2.0–5.0).

Median initial lactate values were higher in the non-survivors compared to the survivors, 4 mmol/L (IQR 3.0–8.5) vs. 2 mmol/L (IQR 2.0–4.0), $p < 0.001$.

After adjusting for age, gender, renal disease, malignancy, and SOFA scores, initial POC lactate was independently associated with increased in-hospital mortality [OR 1.41 95% CI (1.06, 1.87) $p = 0.018$], as shown in Table 2.

3.3. Optimal lactate cutoff that best predicted in-hospital mortality

Initial lactate was found to have an area under the curve (AUC) of 0.752 (95% CI, 0.643 to 0.86) comparable to SOFA scores with AUC of 0.680 (95% CI, 0.565–0.794) as shown in Figures 1, 2, respectively.

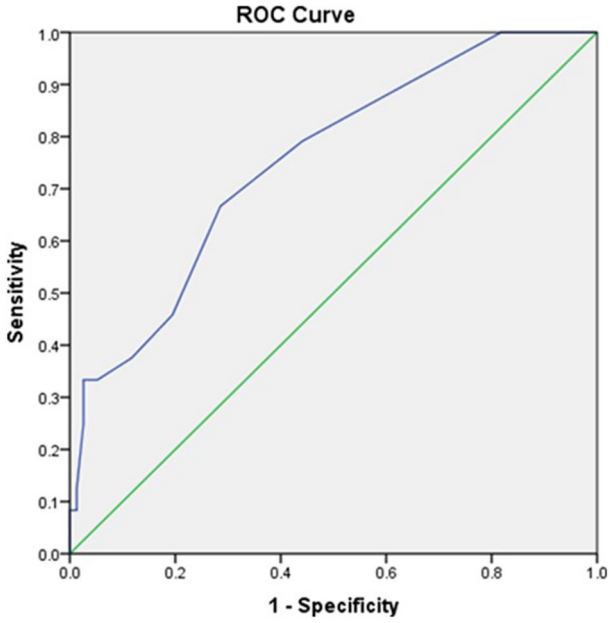

FIGURE 1
The receiver operator curve addressing the association between initial POC GEM lactate values and mortality (AUC = 0.752).

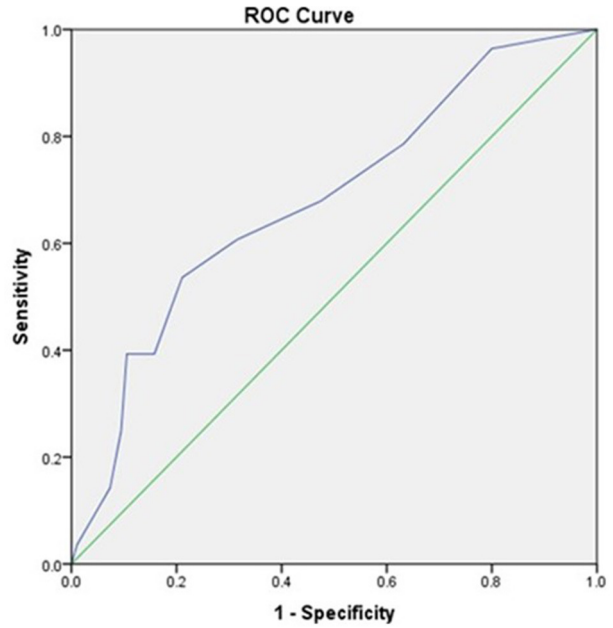

FIGURE 2
The receiver operator curve addressing the association between SOFA Scores and mortality (AUC = 0.680).

An initial lactate cutoff of 3.5 mmol/L was found to best predict in-hospital mortality with a sensitivity and specificity of 66.7 and 71.4%, respectively, as shown in Table 3. The positive predictive value and negative predictive values were 70 and 68.2%, respectively. A cutoff of 2 mmol/L was found to have a lower specificity at 55.8%.

TABLE 3 POC lactate cutoffs and predicting for in-hospital mortality.

Lactate score	Sensitivity	1-Specificity	Specificity	Youden Index
0.5	1	0.987	0.013	0.013
1.5	1	0.818	0.182	0.182
2.5	0.792	0.442	0.558	0.35
3.5	0.667	0.286	0.714	0.381
4.5	0.458	0.195	0.805	0.263
5.5	0.375	0.117	0.883	0.258
6.5	0.333	0.052	0.948	0.281
7.5	0.333	0.026	0.974	0.307

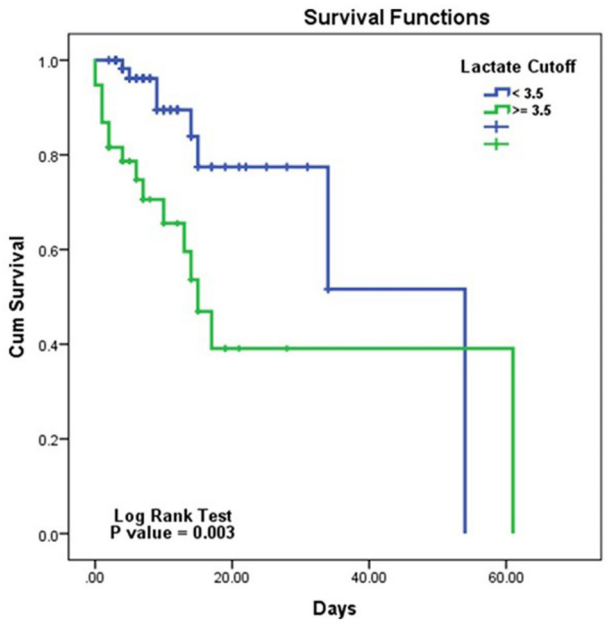

FIGURE 3
Kaplan–Meier survival curves for overall survival according to the lactate cutoff.

Mortality was 42.1% (16/38) in patients with initial lactate of ≥ 3.5 mmol/L and 12.7% (8/63) in patients with initial lactate of <3.5 mmol/L (HR, 3.388; 95% CI, 1.432–8.018; $p < 0.005$) (Figure 3).

4. Discussion

In this study, non-survivors had higher lactate values than survivors. Furthermore, a lactate cutoff of 3.5 mmol/L exhibited the highest diagnostic accuracy for predicting overall in-hospital mortality based on the AUC. Patients with a lactate greater than or equal to 3.5 mmol/L had an in-hospital mortality rate 3.4 times higher than patients with a lactate <3.5 mmol/L.

A cutoff of 2 mmol/L is part of the surviving sepsis campaign's 1 h sepsis bundle and is used to identify those patients with increased mortality risk requiring subsequent lactate measurement and close follow-up. However, the recommendation for the use of a 2 mmol/L cutoff is based on studies using different patient populations, different sample types (arterial, venous, and capillary), and different platforms (15). In our study, a cutoff of 2 mmol/L was found to have a lower specificity in predicting in-hospital mortality compared to a cutoff of 3.5 mmol/L (55.8 vs. 71.4%).

In a retrospective study by Elhouni et al. in South Africa, they evaluated the optimal lactate cutoff for predicting in-hospital mortality in patients admitted with septic shock in the critical care unit. The admission lactate was measured on a blood gas analyzer (GEM 3000). They found an initial lactate cutoff of 4.5 mmol/l to be the most optimal independent predictor of mortality (OR 2.26, 95% CI 1.14–4.52, $p = 0.020$) with an AUC of 0.612 (95% CI 0.527–0.696) (17). Although they used a similar platform to our study, they only evaluated the optimal lactate cutoff in patients with septic shock. Additionally, they had a younger cohort of patients with a median age of 42 years compared to 61 years in this study.

In a prospective cohort study in Uganda, a POC lactate cutoff was determined in HIV-positive patients presenting to the emergency department with sepsis. This was measured on capillary whole blood using a portable handheld POC lactate device (Accutrend portable lactate analyzer). A lactate cutoff of 4.0 mmol/L was identified to have the highest AUC at 0.81 in predicting in-hospital mortality in this cohort of patients (18).

In a prospective cohort study in Tanzania, a POC lactate cutoff was determined in unselected critically ill patients including patients with suspected sepsis presenting to the emergency department. This was venous whole blood tested on a portable cartridge-based POC lactate device (iSTAT Abbott). A lactate cutoff of greater than or equal to 3.8 mmol/L was found to have the highest AUC at 0.80 in predicting in-hospital mortality (19).

The differences in the lactate cutoffs may be due to the different study cohorts. Additionally, the analytical method used may impact the result given the lack of method standardization (22).

The prognostic accuracy of an initial lactate result and SOFA scores were found to be comparable in this study [AUC 0.752, 95% CI (0.643 to 0.86) vs. AUC 0.680, 95% CI, (0.565–0.794)]. This finding was similar to a retrospective study carried out by Liu et al. in China in which they found lactate AUC to be comparable to that of SOFA in patients with sepsis [AUC 0.664, (95% CI, 0.639–0.689) vs. AUC 0.686, (95% CI, 0.661–0.710)] (23). Similarly, in a retrospective study in patients with sepsis secondary to community-acquired pneumonia, the prognostic accuracy of admission lactate was comparable to that of the SOFA score [AUC 0.679, 95% CI (0.612–0.745) vs. AUC 0.795, 95% CI (0.740–0.850)] (24).

There has been a push to enhance the clinical characterization of sepsis in the absence of a gold standard diagnostic test. The SOFA score has been widely validated as the clinical operationalization of sepsis. Lactate is not part of the SOFA score but is used in sepsis algorithms to aid in risk stratification. From this study, initial POC lactate independently predicts in-hospital mortality. Additionally, a single reading of POC lactate is comparable to the SOFA score in predicting in-hospital mortality. This offers an advantage in that a lactate result can be obtained rapidly with a fast turnaround time allowing for the implementation of the 1 h sepsis bundle (6). Conversely, SOFA score parameters are time-consuming and require a well-equipped laboratory making it difficult to use in contact with patients presenting to the emergency department with

sepsis (12). With this in mind, Quick SOFA (qSOFA) was created and noted to have a predictive validity similar to that of the SOFA score out of the critical care setting (5). In our facility, SOFA and not qSOFA are used in the sepsis protocol, and therefore, qSOFA was not evaluated (20).

The most common comorbidity in this cohort of patients was hypertension followed by malignancy and diabetes. Patients with underlying malignancies had overall poorer survival. This was in keeping with a prevalence study carried out by Rhee et al. in a study cohort of sepsis patients from six US hospitals where the most common underlying comorbidity was malignancy which was associated with poorer survival (25). Malignancy as a risk factor for poor outcomes in sepsis is well established due to the underlying immunosuppression (26).

Patients in whom a focus of infection was identified had a better outcome compared to those in whom a focus was not identified [82.1 vs. 42.9%, respectively ($p = 0.011$)]. However, of the 29 patients in whom an outcome was not identified, 55% had an underlying malignancy. This may have resulted in the overall poor survival of these patients. One of the cornerstones of the management of patients with sepsis and septic shock is controlling the source of infection (27). However, this can be challenging in part due to culture-negative sepsis. In a retrospective single-center study in the US, of the patients admitted with sepsis or septic shock over a 7-year period, 89% had culture-negative sepsis (28).

This study has several limitations. This was a single-center study in a private tertiary facility and thus may not be generalizable to other hospitals in Kenya, given possible differences in patient characteristics, management, and availability of resources.

The HR for the identified cutoff had a broad confidence interval. This was due to the small sample size obtained for the study. Additionally, the cutoff identified is valid only for whole blood lactate measured on a GEM blood gas analyzer. Since this measurement is not standardized, it limits the usefulness of the cutoff in sites not using the GEM instrument.

Data were not collected on whether any patient management, such as intravenous fluids or administration of antibiotics, was performed before obtaining the POC GEM lactate result, as this could alter baseline lactate values. However, our data provide evidence from a real-life setting where lactate levels are used to make timely decisions on the management of patients suspected to have sepsis.

Another limitation is that we only focused on in-hospital mortality and did not have data on the outcome of discharged patients including re-admission or transfer to other hospitals. However, mortality as an endpoint is a good indicator of the potential clinical impact of an intervention.

A further limitation is the possible reagent lot-to-lot variability with POC GEM lactate testing. However, any major variability in reagent performance would have been picked up by the daily internal quality control as well as the external quality assurance done periodically.

5. Conclusion

Initial lactate was found to independently predict in-hospital mortality and was found to be comparable to a SOFA score. An initial POC lactate of 3.5 mmol/L best predicted in-hospital mortality in this cohort of patients. This lactate cutoff will be a useful bedside tool for screening and rapidly stratifying patients with suspected sepsis presenting at the emergency department at risk for adverse outcomes. A review of the sepsis and septic shock protocols may help in the early identification and management of these patients to reduce in-hospital mortality at AKUHN.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Aga Khan University Hospital Institutional Scientific and Ethics Review Committee. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

BG, DM, GO, and BW conceptualized the study. BG collected data and drafted the manuscript. JS, DM, and GO conducted data analysis and interpretation. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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