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The emergence of carbapenemase-producing *Enterobacterales* in hospitals: a major challenge for a debilitated healthcare system in Lebanon

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Background: Carbapenem- and extended-spectrum cephalosporin-resistant *Enterobacterales* (CR-E and ESCR-E, respectively) are increasingly isolated worldwide. Information about these bacteria is sporadic in Lebanon and generally relies on conventional diagnostic methods, which is detrimental for a country that is struggling with an unprecedented economic crisis and a collapsing public health system. Here, CR-E isolates from different Lebanese hospitals were characterized.

Materials and methods: Non-duplicate clinical ESCR-E or CR-E isolates ($N = 188$) were collected from three hospitals from June 2019 to December 2020. Isolates were identified by MALDI-TOF, and their antibiotic susceptibility by Kirby-Bauer disk diffusion assay. CR-E isolates ($n = 33/188$) were further analyzed using Illumina-based WGS to identify resistome, MLST, and plasmid types. Additionally, the genetic relatedness of the CR-E isolates was evaluated using an Infrared Biotyper system and compared to WGS.

Results: Using the Kirby-Bauer disk diffusion assay, only 90 isolates out of the 188 isolates that were collected based on their initial routine susceptibility profile by the three participating hospitals could be confirmed as ESCR-E or CR-E isolates and were included in this study. This collection comprised *E. coli* ($n = 70$; 77.8%), *K. pneumoniae* ($n = 13$; 14.4%), *Enterobacter* spp. ($n = 6$; 6.7%), and *Proteus mirabilis* ($n = 1$; 1.1%). While 57 were only ESBL producers the remaining 33 isolates (i.e., 26 *E. coli*, five *K. pneumoniae*, one *E. cloacae*, and one *Enterobacter hormaechei*) were resistant to at least one carbapenem, of which 20 were also ESBL-producers. Among the 33 CR-E, five different carbapenemase determinants were identified: bla_{NDM-5} (14/33), $bla_{OXA-244}$ (10/33), bla_{OXA-48} (5/33), bla_{NDM-1} (3/33), and $bla_{OXA-181}$ (1/33) genes. Notably, 20 CR-E isolates were also ESBL-producers. The analysis of the genetic relatedness revealed a substantial genetic diversity among CR-E isolates, suggesting evolution and transmission from various sources.

Conclusion: This study highlighted the emergence and broad dissemination of bla_{NDM-5} and $bla_{OXA-244}$ genes in Lebanese clinical settings. The weak AMR awareness in the Lebanese community and the ongoing economic and healthcare challenges have spurred self-medication practices. Our findings highlight an urgent need for transformative approaches to combat antimicrobial resistance in both community and hospital settings.

1 Introduction

The order Enterobacterales includes the most common human bacterial pathogens responsible for community- and healthcare-associated infections. These species have the ability to rapidly evolve through horizontal gene transfer (e.g., mobile genetic elements) (1). This includes the ability to develop resistance to multiple antibiotics, which complicates the treatment of infections and increases, potentially mortality and morbidity in patients. Of particular concern is the emergence of carbapenem-resistant Enterobacterales (CR-E), which poses a major concern in clinical as well as community settings all across the globe (2). Carbapenems referred to as last-resort antibiotics, possess a broad spectrum of activity against most clinically-relevant Gram-negative bacteria (3, 4). Consequently, it is crucial to continuously monitor and assess the spread of carbapenem resistance, especially in low- and middle-income countries (LMICs) that face established challenges in antimicrobial stewardship and public health systems.

Available observations suggest that antimicrobial resistance (AMR) has been precipitously increasing in Lebanon, a country with a plethora of issues resulting from an unprecedented economic collapse (5–7). The latter has amplified critical issues such as access to medical care, sanitation, and nutritious and safe food, as well as promoted lax medical practices, including self-medication and the reliance on widely and easily available antibiotics as cheaper alternatives across the country. This is important because excessive and inappropriate use of antimicrobials in human and veterinary medicine and agriculture has been well-documented in Lebanon, even before the economic collapse (8–10). Taken together, these challenging conditions have been predicted to enhance the emergence of AMR and the cycle of complicated infections, especially in the most vulnerable populations in Lebanon (5–7, 10, 11). Nevertheless, studies on AMR in Lebanon are generally scant and, when available, can be limited, in scope or (e.g., low sample number) and/or descriptive (e.g., phenotypic AMR evaluation and absence of in-depth genomic analysis). Despite this, available studies have reported rates of extended-spectrum cephalosporin-resistant Enterobacterales (ESCR-E; ~50% of tested isolates) and CR-E (~3%) among clinical isolates in Lebanon between 2015 and 2019 (12, 13). The enzymatic nature of the carbapenem-resistance has also been evidenced, by the detection of several carbapenemase genes, including bla_{OXA-48}, bla_{NDM-5}, and bla_{NDM-19}, in *Escherichia coli*, bla_{OXA-48}, $bla_{OXA-181}$, and bla_{NDM-5} in *Klebsiella pneumoniae*, bla_{OXA-48}, bla_{VIM-1}, bla_{VIM-4}, and bla_{NDM-1} in *Enterobacter cloacae*, and bla_{OXA-48} in *Citrobacter freundii* isolated in Lebanese hospitals (12). In addition, high rates of multidrug-resistant

(MDR) Enterobacterales (60.7%) and the dissemination of extended-spectrum ß-lactamase (ESBL) producing-, carbapenemase-producing (CP)-, and colistin-resistant *E. coli* isolates among healthy people in the Lebanese community (14), animals (15), and the environment (16) has been documented. Hence, in-depth studies to evaluate the emergence and spread of AMR in Lebanon, focusing on the determinants that contribute to the dissemination of ESBL-E and/or CP-E is now mandatory. Specifically, details of the sequence types of these bacteria, their resistome, and the plasmids carrying these genes may be crucial to identify the transmission routes and propose intervention strategies to limit their spread. To fill these gaps, we evaluated the occurrence of ESBL- and/or CP-E in three different hospitals in Lebanon and determined the underlying mechanisms of resistance, the population structure of the isolates, and the associated plasmid types using whole genome sequencing analysis (WGS), a powerful technique for investigating AMR, but still not commonly available in LMICs, such as Lebanon, due to its relative expensiveness and requirement of specialized equipment and skills (17).

This study focused only on hospital isolates, as (1) anecdotal evidence suggests that hospitals play an important role in the transmission of ESBL-E- and CP-E, and (2) hospitalized patients are more susceptible to infections/colonization with these species.

2 Materials and methods

2.1 Ethical approval

This study was approved by the Azm Center/Lebanese University ethical committee and the Lebanese Ministry of Public Health (CE-EDST-1-2020). All the specimens were analyzed anonymously, without any patient identifiers, and the patients were not physically involved in this study.

2.2 Isolation and identification of bacteria from clinical samples

A total of 188 clinical Enterobacterales isolates being either resistant to Expanded Spectrum Cephalosporin (ESCR) or Carbapenem Resistant were collected between 2019 and 2020 by the bacteriology laboratories of three hospitals, including El Youssef Hospital Center (50 isolates), the Nini Hospital (137 isolates), and the Tripoli Governmental Hospital (1 isolate), which are located in the Akkar and North governorates of Lebanon, respectively. These isolates

were collected based on their susceptibility profile established in the hospitals as part of routine clinical testing. These clinical isolates were recovered from different sample types, including urine, pus, wound, rectal, axillary, pleural fluid, gastric fluid, and bronchial fluid. They were identified at the hospitals using the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) with VITEK MS protocol (bioMérieux, Version 3.0, Marcy L'Etoile, France). The isolates were subsequently stored at the Lebanese University bacterial bank (CMUL).

2.3 Antimicrobial susceptibility testing

The isolates were screened for ESBL and CR phenotypes using the Kirby-Bauer disk diffusion assay (including Ticarcillin, Ticarcillin/clavulanic acid, cefoxitin, ceftazidime, cefepime, temocillin, and imipenem). As for the CR-E isolates, a total of 15 β-lactams (amoxicillin, amoxicillin/clavulanic acid, ticarcillin, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, cefoxitin, cefotaxime, cefepime, ceftazidime, aztreonam, ertapenem, imipenem, meropenem, and temocillin) antibiotics of human interest were tested. The minimum inhibitory concentrations (MICs) were also determined by E-test (bioMérieux) for ertapenem, imipenem, and meropenem, while temocillin and colistin MICs were assessed using the broth microdilution method.

Additionally, 13 CP-*E. coli* isolates were selected according to their resistance phenotype, genotype, and MLST type for further determination of MIC values using broth microdilution (BMD) test (Sensititre, ThermoFisher, Grenoble, France) for a complementary list of novel beta-lactam and non-beta-lactam antibiotics of clinical and veterinary interest (i.e., ceftazidime/avibactam, ceftiofur, ceftaroline, ceftobiprole, aztreonam-avibactam, mecillinam, imipenem/relebactam, meropenem/vaborbactam, cefiderocol, eravacyclin, apramycin, gentamicin, neomycin, streptomycin, sulfonamides, and nitrofurantoin). Susceptibility patterns were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[1] guidelines when available (18). For aztreonam/avibactam, interpretation was done using aztreonam breakpoints alone (18). For ceftiofur, apramycin, neomycin and streptomycin veterinary breakpoints were used.[2]

2.4 Evaluation of the ESCR- and CR-E isolates using enzymatic assays

The β LACTA™ test (Bio-Rad, Marne-la-Coquette, France) was used to further evaluate the ESCR-E isolates. Briefly, this test is based on the hydrolysis of a chromogenic cephalosporin that turns red upon hydrolysis. Notably, the chromogenic cephalosporin is not hydrolyzed by acquired penicillinases (e.g., SHV-1, TEM-1) but by ESBL, carbapenemase, and acquired AmpC (19). Furthermore, the NG-Test® CTX-M MULTI (NG Biotech, Guipry, France)

immunochromatographic assay (ICA) was performed on the β LACTA™ positive isolates to infer the presence of CTX-M-type ESBLs (20). The CR-E isolates were also evaluated using the Carba NP hydrolysis test, which detects carbapenemase activity based on *in vitro* hydrolysis of imipenem (21). The NG-Test® CARBA-5 ICA (NG Biotech) was used to detect members of the five main families of carbapenemases (i.e., KPC-, NDM-, VIM-, IMP-, and OXA-48-like enzymes) produced by the CR-E isolates as described in the manufacturers' instructions (22).

2.5 Molecular characterization of the ESCR- and CR-E isolates

The ESCR-E isolates (positive using the β-LACTA™ and NG-Test® CTX-M MULTI tests) were screened by PCR and subsequent Sanger sequencing to identify the bla_{CTX-M} allele as described previously (23, 24). For the ESCR-E isolates (positive using the β-LACTA™ but negative with the NG-Test® CTX-M MULTI) and the CR-E isolates, the total DNA was extracted using the PureLink™ Genomic DNA Mini-Kit (ThermoFisher Scientific, Waltham, MA, United States) following the manufacturer's instructions and stored at -20°C. Genomic DNA was used for library preparation using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB, France) according to the manufacturer's instructions. Whole genome sequencing was performed on an Illumina NextSeq 500 instrument (Illumina). After sequencing, raw data were assembled *de novo* using the CLC genomics 10.2 program (Qiagen, Les Ulis, France), and the genomes were analyzed online using software available at the Center for Genomic Epidemiology-CGE.[3] The latter included multilocus sequence typing (MLST) with the CGE MLST 2.0 software to determine sequence types (ST), and acquired resistance gene determinations using ResFinder 4.1 (25–27). Similarly, plasmid replicon types and virulence genes were identified using PlasmidFinder 2.0 (26, 28) and VirulenceFinder 2.0., respectively (26, 29, 30). Reference plasmids sequence were retrieved from the NCBI database, using a local BLAST algorithm. Reads and/or Contigs carrying carbapenemase genes were mapped to reference plasmids, using the CLC genomics 10.2 program (Qiagen).

2.6 Plasmids from CR-E isolates

Plasmids were extracted from the carbapenem-resistant isolates by the Kieser method as previously described (31). Transfer of plasmid-borne resistance markers was assessed by electroporation of the plasmids into electro-competent *E. coli* TOP10 (Invitrogen, Saint-Aubin, France). Transformants were selected on Trypticase soy agar (TSA) supplemented with ticarcillin (100 μg/mL). Transformants were PCR screened for the carbapenemase genes likely transferred to the recipient *E. coli*. Plasmids were visualized using electrophoresis on 0.7% agarose. *E. coli* NCTC 50192, which harbors four plasmids (7, 48, 66, and 154 kb) was used as size markers during electrophoresis (31).

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1 https://www.eucast.org/clinical_breakpoints

2 https://www.sfm-microbiologie.org/wp-content/uploads/2021/12/CASFM_VET2021.pdf

3 https://cge.food.dtu.dk/services/ResFinder/

2.7 Typing of the CR-E isolates using infrared spectrometry

The CR-E isolates were typed using the Bruker IR Biotyper spectrometer (IRBT, Bruker, Hamburg, Germany). An amount of 1 μL of bacterial colonies selected from the confluent part of the culture was re-suspended in 50 μL of 70% ethanol solution in an IR Biotyper suspension vial. After vortexing, 50 μL of deionized water was added, and the solution was mixed by pipetting. The bacterial suspensions (15 μL) were spotted in three technical replicates onto the 96-spot silicon IR Biotyper target and let dry for 15–20 min at 35°C ± 2°C. In each run, prior to sample spectra acquisition, quality control was performed with the Infrared Test Standards (IRTS 1 and 2) of the IR Biotyper kit. IRTS 1 and IRTS 2 were re-suspended in 90 μL deionized water and 90 μL of absolute ethanol was added and mixed. Subsequently, 12 μL of the suspension was spotted onto the IR Biotyper target and let dry as previously described. The relationships between the isolates were analyzed using the Bruker IR Biotyper Software (version 2.1.0.195, Bruker) (32). An online tool[4] was used to assess the quantitative data of discriminatory power and concordance of the typing methods. Simpson's index of diversity (SID) was used to evaluate the discriminatory power of the typing method, calculating the probability that two unrelated isolates from the test strain set will be clustered into different typing groups. Adjusted Rand index (ARI) with 95% confidence intervals was used to evaluate the concordance of IRBT typing results (33).

3 Results

3.1 Bacterial isolates

Based on the susceptibility profile (Resistance to ESC and/or carbapenems) derived from the microbiology laboratories of the three participating hospitals, 188 isolates, *E. coli* (*n* = 151; 80.3%), *K. pneumoniae* (*n* = 20; 10.6%), *Enterobacter* spp. (*n* = 7; 3.7%), *Proteus mirabilis* (*n* = 6; 3.2%), *Salmonella* spp. (*n* = 3; 1.6%), and *Serratia marcescens* (*n* = 1; 0.5%) were collected. Using the Kirby-Bauer disk diffusion assay, only 90 isolates could be confirmed and were included in this study. This collection comprised *E. coli* (*n* = 70; 77.8%), *K. pneumoniae* (*n* = 13; 14.4%), *Enterobacter* spp. (*n* = 6; 6.7%), and *Proteus mirabilis* (*n* = 1; 1.1%). Routine antimicrobial susceptibility testing revealed that out of these 90 isolates, 57 were only ESBL producers (e.g., fully susceptible to carbapenems), while the remaining 33 (i.e., 26 *E. coli*, five *K. pneumoniae*, one *E. cloacae*, and one *Enterobacter hormaechei*) were resistant to at least one carbapenem, of which 20 were also ESBL-producers, as revealed by synergy images between ESCs and clavulanic acid containing disks.

3.2 Susceptibility testing and ESBL gene characterization among the ESBL-producers

The 57 ESBL-producing isolates (Table S1) were composed of *E. coli* (*n* = 44; 77.2%), *K. pneumoniae* (*n* = 8; 14%), *Enterobacter* spp.

(*n* = 4; 7%), and *Proteus mirabilis* (*n* = 1; 1.8%). These isolates were MDR and remained consistently susceptible only to carbapenems.

The NG-Test® CTX-M MULTI showed that all but two isolates were CTX-M positive (Table S1). PCR amplification of the entire *bla*CTX-M-gene and subsequent sequencing revealed that *bla*CTX-M-15 was predominant (91.2%, 52/55), followed by *bla*CTX-M-55 (3.5%, 2/55) and *bla*CTX-M-3 gene (1.8%, 1/55). The whole genome sequencing analysis showed that the two non-CTX-M producing ESBL isolates: one *E. coli* isolate harboring a *bla*SHV-12 ESBL gene and one *K. pneumoniae* isolate harboring a chromosomally encoded *bla*SHV-187 gene.

3.3 Carbapenemase detection and susceptibility testing of CR-E isolates

The Carba NP hydrolysis test and the NG-Test® CARBA-5, are displayed in Table 1 and Table S2. Among the 33 CR-E isolates, 23 were positive using the Carba NP test, while the remaining isolates were repeatedly negative. Using the NG-Test® CARBA-5 ICA, all 33 isolates were CPs: 17 isolates (51.5%) were positive for NDM and 16 (48.5%) for OXA-48-like enzymes. Out of the 26 *E. coli* isolates different AMR phenotypes were observed (Figure S1). NDM (*n* = 14) and OXA-48-like (*n* = 12) were the only carbapenemases detected in these isolates. Three *K. pneumoniae* isolates were positive for OXA-48-like, and two for NDM. The two *E. cloacae* complex isolates produced either an OXA-48-like or an NDM.

Using disk diffusion antibiograms, all CR-E isolates were found to be resistant to nearly all antibiotics tested routinely in Lebanon, including ertapenem (*n* = 33; 100%). However, 31% (8/26) of these *E. coli* isolates were found susceptible to ertapenem (i.e., MIC ≤ 0.5 mg/L) using the broth microdilution method; of them, six isolates were negative with the Carba NP test, while all were positive by ICA for OXA-48. While NDM- and some OXA-48-like- producing *E. coli* displayed high MIC levels for temocillin, surprisingly most isolates (75%, 9/12) carrying *bla*OXA-48-like had relatively low MICs (≤ 64 μg/mL) for this antibiotic (as compared to 14.3%; 2/14) of the NDM-producing *E. coli* isolates.

Colistin MIC results revealed that almost all CR-E isolates (97%, 32/33) remained consistently susceptible to this antibiotic (MIC ≤ 2 mg/L). Furthermore, the activity of different last-resort beta-lactam and non-beta-lactam antibiotics was assessed against a subpopulation of CR-*E. coli* isolates (6 *bla*NDM and 7 *bla*OXA-48-like-producers). All the tested isolates were resistant to ceftaroline and ceftobiprole but susceptible to eravacyclin, nitrofurantoin, apramycin, and tigecycline. Resistance to cefiderocol, ceftazidime/avibactam, imipenem/relebactam, and meropenem/vaborbactam was observed among the *bla*NDM-producing isolates (Table 2). Additionally, increased MICs to aztreonam/avibactam (4, 8, and 16 mg/L) were noticed among *bla*NDM-producing *E. coli* isolates.

The whole genome sequencing of the 33 CR-E isolates was performed. After read assembly, the contigs were submitted to ResFinder 4.0. In total, 47 different resistance genes were identified (Table S3), which code for resistance determinants to clinically-important classes of antibiotics, including β-lactams, aminoglycosides, tetracycline, quinolones, trimethoprim-sulfamethoxazole, fosfomycin, and sulfonamides. Among *E. coli* isolates, the most frequently identified carbapenemase was NDM-5 (53.8%; 14/26), followed by OXA-244 (38.5%; 10/26), OXA-181 (3.8%; 1/26), and OXA-48 (3.8%,

TABLE 1 Antibiotic susceptibility patterns using Kirby-Bauer disk diffusion of carbapenemase-producing Enterobacterales (expect colistin).

Isolate code	Sample	Hospital	CarbaNP	NG-Test Carba5	β LACTA	NG-Test CTX-M MULTI[1]	FOX	CAZ	CTX	ATM	FEP	IMP	MEM	FDC	MEC	FSF	TGC	CIP	LVX	CHL	SXT	FUR	TMN	AMK	GMN	CST
Escherichia coli																										
O84C6	Urine	YHC	+	OXA-like	+	−	R	R	R	R	R	S	S	S	S	S	S	S	S	R	R	S	S	S	S	2
O84C10	Gastric fluid	YHC	−	OXA-like	+	+	S	R	R	R	R	S	S	S	R	S	S	S	S	S	R	S	S	S	S	4
O84D2	Urine	YHC	+	OXA-like	−	ND	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	1
O84D6	Urine	YHC	−	OXA-like	+	+	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	S	S	S	S	1
O84E5	Urine	YHC	−	OXA-like	−	ND	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	1
O84E7	Urine	YHC	+	NDM	−	ND	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	R	R	R	2
O84E8	Urine	YHC	−	OXA-like	−	ND	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1
O84E9	Urine	NH	+	NDM	+	+	R	R	R	R	R	R	S	R	R	S	S	R	R	R	R	S	R	R	R	2
O84F2	Urine	YHC	−	OXA-like	+	+	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1
O84F5	Urine	YHC	+	NDM	+	−	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S	S	S	0.5
O86D2	Urine	YHC	+	NDM	+	+	R	R	R	R	R	R	R	S	R	S	S	R	R	R	R	S	R	S	R	1
O84F9	Urine	YHC	+	NDM	−	ND	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	S	S	S	S	1
O84G8	Urine	YHC	−	OXA-like	−	ND	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	1
O85A1	Urine	YHC	−	OXA-like	+	+	S	R	R	R	R	S	S	S	S	S	S	R	S	R	S	S	R	S	R	1
O85A3	Urine	NH	−	OXA-like	−	ND	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	1
O85C3	Urine	YHC	+	NDM	+	+	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	R	R	R	2
O85C6	Urine	YHC	+	NDM	+	+	R	R	R	R	R	R	R	R	S	S	S	R	R	S	R	S	S	S	S	2
O85C10	Urine	YHC	+	NDM	+	+	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	R	R	R	S	0.5
O85D6	Pleural fluid	NH	+	NDM	−	ND	R	R	R	S	R	R	R	R	R	S	S	R	R	S	R	S	S	S	S	1
O85D8	Urine	YHC	−	OXA-like	−	ND	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	0.5
O85E2	Axillary	NH	+	NDM	−	ND	R	R	R	S	R	R	R	R	S	S	S	R	R	S	R	S	S	S	S	2
O85F3	Pus	NH	+	NDM	−	ND	R	R	R	S	R	R	R	R	S	S	S	R	R	S	R	S	S	S	S	1
O85G1	Wound	NH	+	NDM	−	ND	R	R	R	S	R	R	R	S	R	S	S	R	R	S	R	S	S	S	S	2
O86A2	Urine	NH	−	OXA-like	−	ND	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	2
O85C4	Urine	NH	+	NDM	+	+	R	R	R	R	R	R	R	R	S	S	S	R	R	R	R	S	R	S	S	1
O86A6	Rectal	NH	+	NDM	+	+	R	R	R	R	R	R	R	R	S	S	S	R	R	S	R	S	R	S	S	1

(Continued)

TABLE 1 (Continued)

Isolate code	Sample	Hospital	CarbaNP	NG-Test Carba5	β LACTA	NG-Test CTX-M MULTI[I]	FOX	CAZ	CTX	ATM	FEP	IMP	MEM	FDC	MEC	FSF	TGC	CIP	LVX	CHL	SXT	FUR	TMN	AMK	GMN	CST
Klebsiella pneumoniae																										
O84C9	Blood culture	TGH	+	NDM	+	+	R	R	R	S	R	S	R	R	R	S	S	S	S	S	R	S	R	R	R	1
O84D5	Pus	YHC	+	OXA-like	+	−	R	R	R	R	R	S	R	S	R	R	S	S	S	R	S	R	R	R	R	1
O84E1	Gastric fluid	YHC	+	OXA-like	+	+	S	R	R	R	R	S	S	S	S	S	S	S	S	S	S	R	R	R	R	1
O85D10	Urine	NH	+	NDM	+	+	R	R	R	R	R	S	S	S	S	R	S	R	R	R	R	S	R	R	R	2
O85E3	Axillary	NH	+	OXA-like	+	+	R	R	R	R	R	S	R	S	S	R	S	S	S	R	S	S	S	S	S	2
Enterobacter spp.																										
O84D8[II]	Urine	NH	+	NDM	+	−	R	R	R	R	R	S	R	R	R	S	S	R	R	R	R	R	R	R	R	1
O84F3[II]	Urine	YHC	+	OXA-like	+	−	R	R	R	R	R	S	R	S	R	R	S	S	S	R	S	S	S	S	S	1

*Susceptibility results were interpreted according to the EUCAST guidelines All isolates are resistant to amoxicillin, amoxicillin/Clavulanate, Ticarcillin, Ticarcillin/Clavulanate, piperacillin, piperacillin/tazobactam, temocillin, and ertapenem; (18). Gray: Resistant; No color: Susceptible; Abbreviations: FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; FEP, Cefepim; IMP, impipenem; MEM, meropenem; FDC, Cefiderocol; MEC, Mecillinam; FSF, Fosfomycin; TGC, Tigecycline; CIP, ciprofloxacin; LVX, levofloxacin; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; FUR, nitrofurantoin; TMN, tobramycin; AMK, Amikacin; GMN, gentamicin; CST, colistin; YHC, El Youssef Hospital Center; TGH, Tripoli Governmental Hospital; NH, Nini Hospital. Colistin MIC was assessed using the broth microdilution method according to the EUCAST guidelines (18). ᴵThe NG-Test CTX-M MULTI was only performed on positive β LACTA isolates. ᴵᴵO84D8, Enterobacter hormaechei; O84F3, Enterobacter cloacae (as identified by MALDI-TOF MS).

1/26). OXA-48 has also been identified in three *K. pneumoniae* and one *E. cloacae* isolates, while NDM-1 ($n = 3$, 9.1%) was detected in two *K. pneumoniae* and one *E. hormaechei* isolates (Table 2). Notably, two plasmid-encoded cephalosporinases, $bla_{CMY-145}$ and bla_{DHA-1} genes that confer resistance to ESCs were detected in three *E. coli* isolates. ESBL genes such as $bla_{CTX-M-15}$ ($n = 15$), $bla_{CTX-M-27}$ ($n = 1$), bla_{TEM-35} ($n = 1$), bla_{SHV-33} ($n = 1$), bla_{SHV-12} ($n = 2$), bla_{SHV-26} ($n = 1$), were also identified. Specifically, the $bla_{CTX-M-15}$ gene was the most frequently detected in *E. coli* ($n = 11$; including one isolate co-harboring two CTX-M alleles: $bla_{CTX-M-15}$ and $bla_{CTX-M-27}$) and *K. pneumoniae* ($n = 4$) isolates. 16S RNA methylase genes were detected among 6 isolates including 5 bla_{NDM-5}-producing *E. coli* and one bla_{NDM-1}-producing *E. hormaechei* isolate.

MLST analysis using the whole genome sequence showed that the CR-*E. coli* belonged to 11 different STs; including ST69 ($n = 6$ isolates), followed by ST648 ($n = 4$), ST167 ($n = 3$), ST361 ($n = 3$), ST405 ($n = 3$), ST10 ($n = 2$), ST90 ($n = 1$), ST940 ($n = 1$), ST38 ($n = 1$), ST46 ($n = 1$), and ST8881 ($n = 1$). Additionally, the CR-*K. pneumoniae* isolates belonged to four different STs, namely ST35, ST37, ST45, and ST1770. *E. cloacae* and *E. hormaechei* belonged to ST1006 and ST182, respectively (Table S3).

Furthermore, alignment of the *ftsI* gene sequences with that of a wild-type gene (*E. coli* NCTC 9022, accession number LR134237) revealed a four amino-acid insertion in PBP3 after residue 333 in 13 of 14 NDM-5-producing *E. coli* isolates. Two types of insertions were detected: YRIN ($n = 7$) and YRIK ($n = 6$). YRIN insertion was found among isolates with ST167 and ST361, and YRIK insertion was present in isolates with ST648 and ST405. ST90 isolate contained neither YRIN nor YRIK insertions. These 4 AA insertions could be correlated with increased MICs to aztreonam/avibactam (4, 8, and 16 mg/L) and cefiderocol among bla_{NDM-5} producing *E. coli* isolates, as compared to similar isolates lacking a 4 AA insertion (0.06 mg/mL).

3.4 Virulence determinants and plasmids in the CR-*Escherichia coli* isolates

Virulence factors (VFs) in the CR-*E. coli* isolates were identified using the CGE VirulenceFinder 2.0. TraT protein, previously shown to mediate resistance to bacterial killing by serum, was detected in 14 *E. coli* isolates, including a bla_{OXA-48}-positive isolate belonging to ST69. Genes encoding adhesins (*ipfA, fimH, afaA, afaC, afaD, afaE*) were found in five *E. coli* that were positive for $bla_{OXA-244}$ (Table 3). Additionally, *iss* encoding an outer membrane lipoprotein that enhances serum resistance, was detected only in one $bla_{OXA-244}$-positive *E. coli* isolate. The capsular genes, *kpsE* and *kpsM*, were both detected in eight isolates, of which three were bla_{NDM-5}-positive (ST648), and 5 were $bla_{OXA-244}$-positive (ST69). Most VFs were detected in two isolates belonging to ST69.

Using PlasmidFinder 2.1, 9 plasmid replicon types were identified. Specifically, the following plasmid types were detected in the *E. coli* isolates; Col ($n = 9$ isolates), IncFII ($n = 18$), IncX3/X4 ($n = 4$), IncI1-I ($n = 3$), and IncFIA ($n = 11$). Additionally, in the *K. pneumoniae* isolates, IncL ($n = 3$), IncM2 ($n = 1$), and IncFIB ($n = 4$) were detected. The *E. cloacae* and *E. hormaechei* isolates carried IncFIB/II and IncL/X3, respectively (Table 4). Transformants were obtained after Kieser plasmid extraction and electro-transformation for 25/33 isolates. For 8 $bla_{OXA-244}$- producing isolates, even with repeated attempts no plasmids were observed on Kieser gel, and no transformants were

TABLE 2 Antibiotic susceptibility patterns of selected carbapenemase-producing *Escherichia coli* isolates for antibiotics considered last resort molecules using broth microdilution test (Sensititre), and interpreted according to the EUCAST guidelines (18).

Isolate code	MLST	Carbapenemase gene	Amino acids insertion in PBP3	CAZ/AVI	CEF	CFT	BPR	FDC	ATM/AVI[1]	IMP/REL	MER/VAB	ERV	APR	NEO	STR	SA
O84C6	ST940	OXA-181	–	S	R	R	R	S	I	S	S	S	S	S	R	S
O84C10	ST46	OXA-244	–	S	R	R	R	S	S	S	S	S	S	S	R	R
O84D2	ST69	OXA-48	–	S	S	R	R	S	S	S	S	S	S	S	R	R
O84D6	ST10	OXA-244	–	S	R	R	R	S	S	S	S	S	S	S	R	R
O84E7	ST405	NDM-5	YRIK	R	R	R	R	R	I	R	R	S	S	S	R	R
O84E9	ST405	NDM-5	YRIK	R	R	R	R	R	I	R	R	S	S	R	R	R
O84F2	ST38	OXA-244	–	S	R	R	R	S	S	S	S	S	S	S	S	S
O86D2	ST90	NDM-5	–	R	R	R	R	S	S	R	R	S	S	R	R	R
O84F9	ST631	NDM-5	YRIN	R	R	R	R	S	I	R	R	S	S	S	R	R
O85A1	ST8881	OXA-244	–	S	R	R	R	S	S	S	S	S	S	S	R	R
O85C6	ST167	NDM-5	YRIN	R	R	R	R	R	I	R	S	S	S	S	R	R
O85E2	ST648	NDM-5	YRIK	R	R	R	R	R	I	R	R	S	S	S	R	R
O86A2	ST69	OXA-244	–	S	S	R	R	S	S	S	S	S	S	S	S	S

The isolate selection was done according to their resistance phenotype, genotype and MLST type. *Dark gray: Resistant; Light gray: Intermediate non-susceptible; No color: Susceptible; Abbreviations: CAZ/AVI, ceftazidime/avibactam; CEF, Ceftiofure; CFT: ceftarolin; BPR, ceftobiprole; FDC: Cefiderocol; ATM/AVI, aztreonam/avibactam; IMP/REL, imipenem/relebactam; MEM/VAB, meropenem/vaborbactam, ERV, eravacyclin; APR, apramycin; NEO, neomycin; STR, streptomycin; SA, sulfonamides.[1] As no breakpoints are yet available, those of ATM were used.

TABLE 3 Virulence genes of the 26 carbapenemase-producing *E. coli* clinical isolates.

Isolate code	Sample	Carbapenemase gene	MLST type	Adhesion						Siderophore		Serum resistance		Invasive	Capsular			Other genes														
				ipfA	fimH	afaA	afaC	afaD	AfaE	iucC	iutA	traT	iss	eilA	capU	kpsE	kpsMII	fyuA	terC	chuA	irp2	ompT	traJ	hra	iha	sitA	papC	yfcv	csgA	hlyE	cia	cib	sat
O84D6**	Urine	bla_{OXA-244}	ST10																															
O85D8**	Urine	bla_{OXA-244}	ST10																															
O84F2**	Urine	bla_{OXA-244}	ST38																															
O84C10**	Gastric Fluid	bla_{OXA-244}	ST46																															
O84D2**	Urine	bla_{OXA-48}	ST69																															
O84E5**	Urine	bla_{OXA-244}	ST69																															
O84E8**	Urine	bla_{OXA-244}	ST69																															
O84G8**	Urine	bla_{OXA-244}	ST69																															
O85A3*	Urine	bla_{OXA-244}	ST69																															
O86A2*	Urine	bla_{OXA-244}	ST69																															
O86D2	Urine	bla_{NDM-5}	ST90																															
O85C6	Urine	bla_{NDM-5}	ST167																															
O85C4	Urine	bla_{NDM-5}	ST167																															
O85C10	Urine	bla_{NDM-5}	ST167																															
O84F5	Urine	bla_{NDM-5}	ST361																															
O84F9	Urine	bla_{NDM-5}	ST361																															
O86A6	Rectal	bla_{NDM-5}	ST361																															
O84E7*	Urine	bla_{NDM-5}	ST405																															
O84E9*	Urine	bla_{NDM-5}	ST405																															
O85C3**	Urine	bla_{NDM-5}	ST405																															
O85E2*	Axillary	bla_{NDM-5}	ST648																															
O85F3*	Pus	bla_{NDM-5}	ST648																															
O85G1*	Wound	bla_{NDM-5}	ST648																															
O85D6*	Pleural Fluid	bla_{NDM-5}	ST648																															
O85A1**	Urine	bla_{OXA-244}	ST8881																															
O84C6**	Urine	bla_{OXA-181}	ST940																															

Virulence genes were detected using the VirulenceFinder 2.0 online tool available at the Center for Genomic Epideiology-CGE (http://www.genomicepidemiology.org/) (26, 29, 30). Virulence genes were detected using 100% identity as cut-off. *Nini Hospital; **El-Youssef Hospital Center; ***Tripoli Governmental Hospital; Different colors represent different sequence types (STs).

TABLE 4 Carbapenemase gene location was determined by plasmid extraction using Kieser technique and by applying a BLASTN algorithm for carbapenemase-genes-carrying contigs and then these contigs were mapped to reference plasmids using CLC genomics.

Isolate code	MLST type	Carbapenemase gene	Location (chromosomal or plasmid-borne)	IncX3	IncX4	IncFIA	IncFIB	IncFII	Col	IncI1-I	IncL	IncM
Escherichia coli												
O84D6**	ST10	$bla_{OXA-244}$	P									
O85D8**	ST10	$bla_{OXA-244}$	C									
O84F2**	ST38	$bla_{OXA-244}$	C									
O84C10**	ST46	$bla_{OXA-244}$	C									
O84D2**	ST69	bla_{OXA-48}	P									
O84E5**	ST69	$bla_{OXA-244}$	C									
O84E8**	ST69	$bla_{OXA-244}$	C									
O84G8**	ST69	$bla_{OXA-244}$	C									
O85A3*	ST69	$bla_{OXA-244}$	C									
O86A2*	ST69	$bla_{OXA-244}$	C									
O86D2**	ST90	bla_{NDM-5}	P									
O85C6**	ST167	bla_{NDM-5}	P									
O85C4*	ST167	bla_{NDM-5}	P									
O85C10**	ST167	bla_{NDM-5}	P									
O84F5**	ST361	bla_{NDM-5}	P									
O84F9**	ST361	bla_{NDM-5}	P									
O86A6*	ST361	bla_{NDM-5}	P									
O84E7*	ST405	bla_{NDM-5}	P									
O84E9*	ST405	bla_{NDM-5}	P									
O85C3**	ST405	bla_{NDM-5}	P									
O85E2*	ST648	bla_{NDM-5}	P									
O85F3*	ST648	bla_{NDM-5}	P									
O85G1*	ST648	bla_{NDM-5}	P									
O85D6*	ST648	bla_{NDM-5}	P									
O85A1**	ST8881	$bla_{OXA-244}$	C									
O84C6**	ST940	$bla_{OXA-181}$	P									

(Continued)

TABLE 4 (Continued)

Isolate code	MLST type	Carbapenemase gene	Location (chromosomal or plasmid-borne)	IncX3	IncX4	IncFIA	IncFIB	IncFII	Col	IncI1-I	IncL	IncM
Klebsiella pneumoniae												
O84E1**	ST35	bla_{OXA-48}	P				■					
O84D5**	ST35	bla_{OXA-48}	P								■	
O85E3*	ST37	bla_{OXA-48}	P				■	■			■	
O84C9****	ST45	bla_{NDM-1}	P								■	■
O85D10*	ST1770	bla_{NDM-1}	P				■	■	■			
Enterobacter spp.												
O84D8*	ST182	bla_{NDM-5}	P									
O84F3**	ST1006	bla_{NDM-1}	P	■							■	

Plasmid incompatibility groups present in the 33 clinical carbapenemase-producing Enterobacterales isolates, were identified using PlasmidFinder 2.1 online tool available at the Center for Genomic Epidemiology -CGE (http://www.genomicepidemiology.org/) using 99% identity cut-off (26, 28–30). *Nini Hospital; **El-Youssef Hospital Center; ***Tripoli Governmental Hospital; Different colors represent different sequence types (STs).

TABLE 5 Comparison of different typing methods for carbapenemase-producing *Escherichia coli* clinical isolates.

Method	No. of types	Simpson's ID (95% CI)
MLST[a]	7	0.875 (0.813–0.937)
IRBT[b]	11	0.933 (0.857–1.000)
WGS[c]	12	0.967 (0.935–0.998)

Simpson's index of diversity used to determine the discriminatory power and concordance of the three typing methods was determined using the online tool 690 (www.comparingpartitions.info). [a]MLST: Multi-locus sequence typing; [b]IRBT: IR Biotyper; [c]WGS: Whole-genome sequencing.

obtained suggesting a chromosomal location. For *K. pneumoniae* and *E. hormaechi*, the bla_{OXA-48} gene was carried on an IncL plasmid.

3.5 Genetic relatedness as revealed by IR Biotyper in comparison to WGS

Sixteen CR-*E. coli* and the five CR-*K. pneumoniae* isolates positive with the Carba NP test were assessed with the IR Biotyper for strain typing and the results were compared with those obtained by WGS analysis (Figures S2, S3). Overall IRBT results corroborated WGS for the typing of the five CR *K. pneumoniae*, which were classified into four IR types (Figure S2). However, IRBT differentiated 16 *E. coli* isolates that belonged to 7 STs into 11 IR types, with 8 IR types comprising only a single isolate (Table 5). The Simpson Index of Diversity (SID) was used to determine the discriminatory power of the typing methods. WGS had the highest discriminatory power (0.967) followed by IRBT (0.933), while the lowest SID was for MLST (0.875). Main discrepancies were observed between IRBT and WGS with *E. coli* ST-648 isolates (O85D6, O85E2, O85G1, and O85F3), which clustered into IR type 7 (Figure S3), but differed from each other by 53, 400, and 1,360 SNIPs.

4 Discussion

The increase in ESCR- and CR-E isolates has been observed in hospitals worldwide. In Lebanon, two nationwide hospital-based retrospective studies documented a high prevalence of MDR pathogens, including ESBL-producing Enterobacterales (34% in 2016) (5). Recently, a community-based study revealed that approximately half of the population carries ESCR-*E. coli* (14), with an important dynamic of acquisition and loss of MDR strains and limited plasmid spread. The occurrence of Enterobacterales with decreased susceptibility to carbapenems, raised from 0.4% in 2008–2010 to 1.6% in 2012 and 3.3% in 2019 in hospitalized patients (12, 35). Despite the concerning increase and reports, there is a lack of molecular data on the epidemiology of ESBL- and CR-E in Lebanon.

Our results highlighted the predominance of the $bla_{CTX-M-15}$ gene (52/57, 91.2%) among ESBL-producing isolates regardless of the co-existence of other ß-lactam resistance determinants, as observed globally (36). CTX-M-15-producing Enterobacterales clones have been widely reported in Lebanon among human, animal, and environmental sources (14, 15, 37–39). The finding that one single *E. coli* isolate may carry two CTX-M (−15 and −27) variants capable

of strongly hydrolyzing ceftazidime and belonging to two different groups of CTX-Ms (Group 1 and 9) is another example of concentration of restriction mechanisms.

Our findings further support the shift from $bla_{\text{OXA-48-like}}$ variants toward $bla_{\text{NDM-5}}$ among *E. coli* in hospital settings in Lebanon (12, 35). OXA-48-like enzymes were the most prevalent carbapenemases among Enterobacterales in Lebanon over the last decade while NDM-5 was uncommon in hospital and community settings (12, 40). In our study, the $bla_{\text{NDM-5}}$ gene has become the predominant carbapenemase gene (14 of 26 *E. coli* isolates). The $bla_{\text{OXA-244 gene}}$ (10/26) came in second place, while $bla_{\text{OXA-48}}$ (1/33), and $bla_{\text{OXA-181}}$ (1/33) genes are rare. OXA-244 producing *E. coli* isolates are increasingly described worldwide (41), and represents a threat to public health because of the difficulties in their detection using classical screening media based on carbapenems and temocillin (42). Indeed, our results confirmed that OXA-244-producing isolates have lower MICs to temocillin and carbapenems as compared to other CP-E, which results in the absence of growth on screening media and thus an underestimation and silent spread. Furthermore, these isolates were negative for the Carba NP test, and only the use of an ICA (here the NG-Test Carba5) revealed a positive signal for OXA-48-like carbapenemase, suggesting that both tests should be used together to maximize the chances of detecting all OXA-48-like carbapenemases. Additionally, the CR *K. pneumoniae* isolates harbored $bla_{\text{OXA-48}}$ (3/5) and $bla_{\text{NDM-1}}$ (2/5), while the *E. cloacae* and *E. hormaechei* isolates harbored $bla_{\text{OXA-48}}$ and $bla_{\text{NDM-1}}$, respectively.

MLST results demonstrated several distinct genetic backgrounds for the CR-E isolates, suggesting horizontal gene transfer of the carbapenemase gene carrying plasmids, particularly those carrying the $bla_{\text{NDM-5}}$ gene, rather than a clonal spread of a single clone. Interestingly, we found that the carbapenemase genes were plasmid-borne (except in eight $bla_{\text{OXA-244}}$-producing *E. coli*) and on different plasmid types (Table 4). Of the NDM-5-producing *E. coli* isolates, six and seven had the insertion of YRIN or YRIK in PBP3, respectively, which resulted in a significant increase of MICs to aztreonam/avibactam and cefiderocol as well as to other PBP3-targeting ß-lactams (34). Surprisingly, the $bla_{\text{OXA-244}}$ gene was chromosomally encoded in 8 isolates, while it was plasmid-mediated in only two isolates belonging to ST69 and ST10. The chromosomal location of the $bla_{\text{OXA-244}}$ gene was previously reported in ST38 and ST69 in France (43). Taken together, the occurrence of different resistance markers in diverse genetic backgrounds as well as plasmid types constitute a risk to patients, potentially highlighting a more severe problem and highlighting an urgent need to monitor and control the spread of resistance in hospitals in Lebanon.

The study identified various MLST types, including ST648, ST167, ST361, and ST405, in $bla_{\text{NDM-5}}$-producing *E. coli*. These STs are recognized as high-risk global clones that contribute to the widespread dissemination of drug resistance determinants among *Enterobacterales* (44). For instance, ST648 is recognized as a major global ESBL-producing *E. coli* clone (45, 46), particularly associated with $bla_{\text{CTX-M-15}}$, in humans, birds, and companion animals (47). Additionally, ST648 was identified in Lebanon among clinical CRE (48), including $bla_{\text{NDM-5}}$-producing *E. coli* (49). Besides, ST167 has been linked to the global spread of bla_{NDM} in humans, animals, and food (50). ST405 has been detected in several countries, including the United States (51), Japan (52), and Lebanon (48), allowing the

transmission of $bla_{\text{CTX-M-15}}$ and *aac(6′)-Ib-cr* genes (53). Notably, both ST361 and ST648 have been reported among Syrian refugees in Lebanon (54), and ST90 was found in the effluent of Al-Qaa refugee camp (55). Additionally, we identified ST38 among $bla_{\text{OXA-244}}$-producing *E. coli*, previously described in estuary water in Lebanon (56). Several European countries have reported increased dissemination of the $bla_{\text{OXA-244}}$ gene (57, 58), with ST38 being the most common sequence type among $bla_{\text{OXA-244}}$-producing *E. coli* isolates (59). Moreover, ST38 $bla_{\text{OXA-48}}$-producing *E. coli* has also been found in fowls in Lebanon (60). As observed in our study, ST940 *E. coli* carrying the $bla_{\text{OXA-181}}$ gene was previously reported at the American University of Beirut Medical Center (61). Among the *K. pneumoniae* isolates, there were five isolates belonging to four different sequence types. One of them, *K. quasipneumoniae* subsp. *similipneumoniae* ST1770, has been previously reported in hospital wastewater effluents in Japan (62), but had not been reported in Lebanon. Additionally, clinical *K. pneumoniae* isolates ST35 and ST45 have been documented in Lebanon (63, 64). ST37 has also been closely associated with ESBLs (65). Furthermore, the ST182 $bla_{\text{NDM-1}}$ *E. hormaechei* isolate, previously reported in Lebanon (66), has been frequently isolated from clinical specimens in China, Mexico, the Czech Republic, and the United States (67).

Although there is a paramount need to monitor the spread of critical AMR strains in hospitals in Lebanon, these efforts are complicated by the unavailability of resources. Molecular typing methods such as whole genome sequencing are relatively time-consuming and expensive (32). Therefore, we evaluated the Bruker IR Biotyper for reliable detection of the relatedness and discrimination between strains. Our findings showed that Fourier Transform Infrared (FT-IR) technology is a powerful tool for strain typing, showing slightly better results to MLST and comparable results to WGS among CR-*E. coli* and *K. pneumoniae* isolates. Its advantages are summed up in the simple preparation of samples, ease of use, and low running costs. Together with its relatively high discriminatory power, the FT-IR seems to be a good tool for outbreaks real-time surveillance and infection control in clinical settings.

In conclusion, our study showed that in-depth studies are crucial to better understand the emergence and dissemination of drug-resistant determinants within and across healthcare institutions. Specifically, we highlighted an unprecedented diversity of ESBL- and CR-E determinants compared to other studies in Lebanon. Furthermore, the MLST and the associated plasmid types suggested that these determinants were circulating in diverse strains, complicating the control efforts and suggesting the need for evidence-based antimicrobial stewardship programs. In the distressing situation of Lebanon, the accessibility to novel antibiotic molecules remains a major concern, impacting public health. The economic crisis had a profound impact leading to significant challenges in antibiotics procurement and even more so to new molecules, which are unavailable and not yet routinely tested. Furthermore, our data support that the fight against MDR bacteria in LMICs, such as Lebanon, requires a comprehensive One Health approach because of the diffuse sources and factors that affect the spread of resistance in hospitals and the community. The latter corroborates our previous research that showed a wide reliance on and sometimes indiscriminate use of critically important antibiotics in healthcare settings, agriculture, and the community in Lebanon (8, 9). This approach and science-based interventions are urgently needed to control the spread

of AMR in Lebanon, which represents an essential threat nationally and globally as AMR is known to spill across international borders.

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 at http://www.ncbi.nlm.nih.gov/bioproject and PRJNA973232.

Ethics statement

The studies involving humans were approved by Azm Center/ Lebanese University ethical committee and the Lebanese Ministry of Public Health (CE-EDST-1-2020). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from bacterial isolates collected based on susceptibility profiles from three hospitals. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

DD: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. SO: Supervision, Validation, Writing – review & editing. MR: Investigation, Writing – review & editing. IK: Investigation, Resources, Writing – review & editing. HM: Investigation, Writing – review & editing. AB: Formal analysis, Validation, Writing – review & editing. DG: Formal analysis, Investigation, Writing – review & editing. MH: Methodology, Writing – review & editing. FD: Formal analysis, Investigation, Writing – review & editing. MO: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – original draft, Writing – review & editing. TN: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing, Project administration, Validation.

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/fpubh.2023.1290912/full#supplementary-material

References

1. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis*. (2011) 17:1791–8. doi: 10.3201/eid1710.110655

2. Matar GM, Andremont A, Bazzi W. Editorial: combating antimicrobial resistance - a one health approach. *Front Cell Infect Microbiol*. (2019) 9:458. doi: 10.3389/fcimb.2019.00458

3. Iovleva A, Doi Y. Carbapenem-Resistant Enterobacteriaceae. *Clin Lab Med*. (2017) 37:303–15. doi: 10.1016/j.cll.2017.01.005

4. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother*. (2011) 55:4943–60. doi: 10.1128/AAC.00296-11

5. Osman M, Kasir D, Kassem II, Hamze M. Shortage of appropriate diagnostics for antimicrobial resistance in Lebanese clinical settings: a crisis amplified by COVID-19 and economic collapse. *J Glob Antimicrob Resist*. (2021) 27:72–4. doi: 10.1016/j.jgar.2021.08.008

6. Kassem II, Osman M. A brewing storm: the impact of economic collapse on the access to antimicrobials in Lebanon. *J Glob Antimicrob Resist*. (2022) 29:313–5. doi: 10.1016/j.jgar.2022.04.023

7. Osman M, Cummings KJ, El Omari K, Kassem II. Catch-22: war, refugees, COVID-19, and the scourge of antimicrobial resistance. *Front Med (Lausanne)*. (2022) 9:921921. doi: 10.3389/fmed.2022.921921

8. Al Omari S, Al Mir H, Wrayde S, Merhabi S, Dhaybi I, Jamal S, et al. First Lebanese antibiotic awareness week campaign: knowledge, attitudes and practices towards antibiotics. *J Hosp Infect*. (2019) 101:475–9. doi: 10.1016/j.jhin.2018.07.009

9. Kassem II, Hijazi MA, Saab R. On a collision course: the availability and use of colistin-containing drugs in human therapeutics and food-animal farming in Lebanon. *J Glob Antimicrob Resist*. (2019) 16:162–4. doi: 10.1016/j.jgar.2019.01.019

10. Osman M, Rafei R, Ismail MB, Omari SA, Mallat H, Dabboussi F, et al. Antimicrobial resistance in the protracted Syrian conflict: halting a war in the war. *Future Microbiol*. (2021) 16:825–45. doi: 10.2217/fmb-2021-0040

11. Kassem II, Osman M, Jaafar H, El Omari K. Refugee settlements, sewage pollution, COVID-19 and the unfolding cholera outbreak in Lebanon. *J Travel Med*. (2022) 29:taac142. doi: 10.1093/jtm/taac142

12. Rima M, Oueslati S, Dabos L, Daaboul D, Mallat H, Bou Raad E, et al. Prevalence and molecular mechanisms of carbapenem resistance among gram-negative bacilli in three hospitals of northern Lebanon. *Antibiotics (Basel)*. (2022) 11:1295. doi: 10.3390/antibiotics11101295

13. Moghnieh R, Araj GF, Awad L, Daoud Z, Mokhbat JE, Jisr T, et al. A compilation of antimicrobial susceptibility data from a network of 13 Lebanese hospitals reflecting the national situation during 2015-2016. *Antimicrob Resist Infect Control*. (2019) 8:41. doi: 10.1186/s13756-019-0487-5

14. Al-Mir H, Osman M, Drapeau A, Hamze M, Madec JY, Haenni M. Spread of ESC-, carbapenem- and colistin-resistant Escherichia coli clones and plasmids within and between food workers in Lebanon. *J Antimicrob Chemother*. (2021) 76:3135–43. doi: 10.1093/jac/dkab327

15. Al-Mir H, Osman M, Drapeau A, Hamze M, Madec JY, Haenni M. WGS analysis of clonal and plasmidic epidemiology of colistin-resistance mediated by *mcr* genes in the poultry sector in Lebanon. *Front Microbiol*. (2021) 12:624194. doi: 10.3389/fmicb.2021.624194

16. Nasser NA, Mann D, Li S, Deng X, Kassem II. Draft genome sequences of colistin-resistant and *mcr-1.1*-carrying Escherichia coli strains isolated from irrigation water. *Microbiol Resour Announc*. (2021) 10:e00120-21. doi: 10.1128/MRA.00120-21

17. Gilchrist CA, Turner SD, Riley MF, Petri WA Jr, Hewlett EL. Whole-genome sequencing in outbreak analysis. *Clin Microbiol Rev*. (2015) 28:541–63. doi: 10.1128/CMR.00075-13

18. CASFM-European Society of Clinical Microbiology and Infectious Disease Guidelines. *Recommandations*. (2023).

19. Renvoisé A, Decré D, Amarsy-Guerle R, Huang TD, Jost C, Podglajen I, et al. Evaluation of the βLacta test, a rapid test detecting resistance to third-generation cephalosporins in clinical strains of Enterobacteriaceae. *J Clin Microbiol*. (2013) 51:4012–7. doi: 10.1128/JCM.01936-13

20. Bernabeu S, Ratnam KC, Boutal H, Gonzalez C, Vogel A, Devilliers K, et al. A lateral flow immunoassay for the rapid identification of CTX-M-producing Enterobacterales from culture plates and positive blood cultures. *Diagnostics (Basel)*. (2020) 10:764. doi: 10.3390/diagnostics10100764

21. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis*. (2012) 18:1503–7. doi: 10.3201/eid1809.120355

22. Boutal H, Vogel A, Bernabeu S, Devilliers K, Creton E, Cotellon G, et al. A multiplex lateral flow immunoassay for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemase-producing Enterobacteriaceae. *J Antimicrob Chemother*. (2018) 73:909–15. doi: 10.1093/jac/dkx521

23. Karim A, Poirel L, Nagarajan S, Nordmann P. Plasmid-mediated extended-spectrum beta-lactamase (CTX-M-3 like) from India and gene association with insertion sequence ISEcp1. *FEMS Microbiol Lett*. (2001) 201:237–41. doi: 10.1111/j.1574-6968.2001.tb10762.x

24. Poirel L, Decousser JW, Nordmann P. Insertion sequence ISEcp1B is involved in expression and mobilization of a *Bla*(CTX-M) beta-lactamase gene. *Antimicrob Agents Chemother*. (2003) 47:2938–45. doi: 10.1128/AAC.47.9.2938-2945.2003

25. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother*. (2020) 75:3491–500. doi: 10.1093/jac/dkaa345

26. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. *BMC bioinformatics*. (2009) 10:421. doi: 10.1186/1471-2105-10-421

27. Zankari E, Allesøe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J Antimicrob Chemother*. (2017) 72:2764–8. doi: 10.1093/jac/dkx217

28. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother*. (2014) 58:3895–903. doi: 10.1128/AAC.02412-14 (Accessed November 2, 2023).

29. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. *J Clin Microbiol*. (2014) 52:1501–10. doi: 10.1128/JCM.03617-13

30. Malberg Tetzschner AM, Johnson JR, Johnston BD. In silico genotyping of Escherichia coli isolates for extraintestinal virulence genes by use of whole-genome sequencing data. *J Clin Microbiol*. (2020) 58:e01269-20. doi: 10.1128/JCM.01269-20

31. Kieser T. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid*. (1984) 12:19–36. doi: 10.1016/0147-619X(84)90063-5

32. Alhasabi J, Balakrishnan I, Wey E. Application of the Bruker IR Biotyper for strain typing during an outbreak. *Access Microbiol*. (2020) 2. doi: 10.1099/acmi.fis2019.po0086

33. Carriço JA, Silva-Costa C, Melo-Cristino J, Pinto FR, de Lencastre H, Almeida JS, et al. Illustration of a common framework for relating multiple typing methods by application to macrolide-resistant *Streptococcus pyogenes*. *J Clin Microbiol*. (2006) 44:2524–32. doi: 10.1128/JCM.02536-05

34. Patino-Navarrete R, Rosinski-Chupin I, Cabanel N, Gauthier L, Takissian J, Madec JY, et al. Stepwise evolution and convergent recombination underlie the global

dissemination of carbapenemase-producing *Escherichia coli. Genome Med*. (2020) 12:10. doi: 10.1186/s13073-019-0699-6

35. Beyrouthy R, Robin F, Dabboussi F, Mallat H, Hamze M, Bonnet R. Carbapenemase and virulence factors of *Enterobacteriaceae* in North Lebanon between 2008 and 2012: evolution via endemic spread of OXA-48. *J Antimicrob Chemother*. (2014) 69:2699–705. doi: 10.1093/jac/dku181

36. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *J Antimicrob Chemother*. (2017) 72:2145–55. doi: 10.1093/jac/dkx146

37. Diab M, Hamze M, Madec JY, Haenni M. High prevalence of non-ST131 CTX-M-15-producing *Escherichia coli* in healthy cattle in Lebanon. *Microb Drug Resist*. (2017) 23:261–6. doi: 10.1089/mdr.2016.0019

38. Al-Mir H, Osman M, Azar N, Madec JY, Hamze M, Haenni M. Emergence of clinical mcr-1-positive *Escherichia coli* in Lebanon. *J Glob Antimicrob Resist*. (2019) 19:83–4. doi: 10.1016/j.jgar.2019.08.019

39. Kassem II, Hassan J, Esseili MA, Mann D, Osman M, Li S, et al. Draft genome sequences of multidrug-resistant *Escherichia coli* isolated from river water. *Microbiol Resour Announc*. (2022) 11:e0082722. doi: 10.1128/mra.00827-22

40. Fadlallah M, Salman A, Salem-Sokhn E. Updates on the status of carbapenem-resistant Enterobacterales in Lebanon. *Int J Microbiol*. (2023) 2023:1–10. doi: 10.1155/2023/8831804

41. Pitout JDD, Peirano G, Kock MM, Strydom K-A, Matsumura Y. The global ascendency of OXA-48-type carbapenemases. *Clin Microbiol Rev*. (2019) 33:e00102-19. doi: 10.1128/cmr.00102-19

42. Rima M, Emeraud C, Bonnin RA, Gonzalez C, Dortet L, Iorga BI, et al. Biochemical characterization of OXA-244, an emerging OXA-48 variant with reduced β-lactam hydrolytic activity. *J Antimicrob Chemother*. (2021) 76:2024–8. doi: 10.1093/jac/dkab142

43. Emeraud C, Girlich D, Bonnin RA, Jousset AB, Naas T, Dortet L. Emergence and polyclonal dissemination of OXA-244-producing *Escherichia coli*. France Emerg Infect Dis. (2021) 27:1206–10. doi: 10.3201/eid2704.204459

44. Mathers AJ, Peirano G, Pitout JD. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin Microbiol Rev*. (2015) 28:565–91. doi: 10.1128/CMR.00116-14

45. Ewers C, Bethe A, Stamm I, Grobbel M, Kopp PA, Guerra B, et al. CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? *J Antimicrob Chemother*. (2014) 69:1224–30. doi: 10.1093/jac/dkt516

46. Peirano G, van der Bij AK, Gregson DB, Pitout JD. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum beta-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. *J Clin Microbiol*. (2012) 50:294–9. doi: 10.1128/JCM.06025-11

47. Liu X, Thungrat K, Boothe DM. Occurrence of OXA-48 carbapenemase and other beta-lactamase genes in ESBL-producing multidrug resistant *Escherichia coli* from dogs and cats in the United States, 2009-2013. *Front Microbiol*. (2016) 7:1057. doi: 10.3389/fmicb.2016.01057

48. Dagher C, Salloum T, Alousi S. Molecular characterization of carbapenem resistant *Escherichia coli* recovered from a tertiary hospital in Lebanon. *PLoS One*. (2018) 13:e0203323. doi: 10.1371/journal.pone.0203323

49. Hodroj Z. *Utilizing NGS in AMR surveillance in North Lebanon - a one health approach* American University of Beirut (2023) 12:3981–85.

50. Baloch Z, Lv L, Yi L, Wan M, Aslam B, Yang J, et al. Emergence of almost identical F36:A-:B32 plasmids carrying *Bla*(NDM-5) and qepA in *Escherichia coli* from both Pakistan and Canada. *Infect Drug Resist*. (2019) 12:3981–5. doi: 10.2147/IDR.S236766

51. Tian GB, Rivera JI, Park YS, Johnson LE, Hingwe A, Adams-Haduch JM, et al. Sequence type ST405 *Escherichia coli* isolate producing QepA1, CTX-M-15, and RmtB from Detroit, Michigan. *Antimicrob Agents Chemother*. (2011) 55:3966–7. doi: 10.1128/AAC.00652-11

52. Matsumura Y, Yamamoto M, Nagao M, Ito Y, Takakura S, Ichiyama S. Association of fluoroquinolone resistance, virulence genes, and IncF plasmids with extended-spectrum-β-lactamase-producing *Escherichia coli* sequence type 131 (ST131) and ST405 clonal groups. *Antimicrob Agents Chemother*. (2013) 57:4736–42. doi: 10.1128/AAC.00641-13

53. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis*. (2008) 14:195–200. doi: 10.3201/eid1402.070350

54. Azour A, Al-Bayssari C, Dagher TN, Fajloun F, Fajloun M, Rolain JM. Clonal dissemination of plasmid-mediated carbapenem and colistin resistance in refugees living in overcrowded camps in North Lebanon. *Antibiotics (Basel)*. (2021) 10:1478. doi: 10.3390/antibiotics10121478

55. Tokajian S, Moghnieh R, Salloum T, Arabaghian H, Alousi S, Moussa J, et al. Extended-spectrum beta-lactamase-producing *Escherichia coli* in wastewaters and refugee camp in Lebanon. *Future Microbiol*. (2018) 13:81–95. doi: 10.2217/fmb-2017-0093

56. Diab M, Hamze M, Bonnet R, Saras E, Madec JY, Haenni M. Extended-spectrum beta-lactamase (ESBL)- and carbapenemase-producing *Enterobacteriaceae* in water sources in Lebanon. *Vet Microbiol*. (2018) 217:97–103. doi: 10.1016/j.vetmic.2018.03.007

57. Falgenhauer L, Nordmann P, Imirzalioglu C, Yao Y, Falgenhauer J, Hauri AM, et al. Cross-border emergence of clonal lineages of ST38 *Escherichia coli* producing the OXA-48-like carbapenemase OXA-244 in Germany and Switzerland. *Int J Antimicrob Agents*. (2020) 56:106157. doi: 10.1016/j.ijantimicag.2020.106157

58. Hammerum AM, Porsbo LJ, Hansen F, Roer L, Kaya H, Henius A, et al. Surveillance of OXA-244-producing *Escherichia coli* and epidemiologic investigation of cases, Denmark, January 2016 to august 2019. *Euro Surveill*. (2020) 25:1900742. doi: 10.2807/1560-7917.ES.2020.25.18.1900742

59. Potron A, Poirel L, Dortet L, Nordmann P. Characterisation of OXA-244, a chromosomally-encoded OXA-48-like β-lactamase from *Escherichia coli*. *Int J Antimicrob Agents*. (2016) 47:102–3. doi: 10.1016/j.ijantimicag.2015.10.015

60. Al Bayssari C, Olaitan AO, Dabboussi F, Hamze M, Rolain JM. Emergence of OXA-48-producing *Escherichia coli* clone ST38 in fowl. *Antimicrob Agents Chemother*. (2015) 59:745–6. doi: 10.1128/AAC.03552-14

61. Bitar I, Dagher C, Salloum T, Araj G, Tokajian S. First report of an *Escherichia coli* from Lebanon carrying an OXA-181 carbapenemase resistance determinant. *J Glob Antimicrob Resist*. (2018) 12:113–4. doi: 10.1016/j.jgar.2018.01.002

62. Takizawa S, Soga E, Hayashi W, Sakaguchi K, Koide S, Tanabe M, et al. Genomic landscape of *Bla*(GES-5)- and *Bla*(GES-24)-harboring gram-negative bacteria from hospital wastewater: emergence of class 3 integron-associated Bla(GES-24) genes. *J Glob Antimicrob Resist*. (2022) 31:196–206. doi: 10.1016/j.jgar.2022.09.005

63. Arabaghian H, Salloum T, Alousi S, Panossian B, Araj GF, Tokajian S. Molecular characterization of carbapenem resistant *Klebsiella pneumoniae* and *Klebsiella quasipneumoniae* isolated from Lebanon. *Sci Rep*. (2019) 9:531. doi: 10.1038/s41598-018-36554-2

64. Nawfal Dagher T, Al-Bayssari C, Chabou S, Baron S, Hadjadj L, Diene SM, et al. Intestinal carriage of colistin-resistant *Enterobacteriaceae* at Saint Georges Hospital in Lebanon. *J Glob Antimicrob Resist*. (2020) 21:386–90. doi: 10.1016/j.jgar.2019.12.001

65. Deng J, Li YT, Shen X, Yu YW, Lin HL, Zhao QF, et al. Risk factors and molecular epidemiology of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in Xiamen. *China J Glob Antimicrob Resist*. (2017) 11:23–7. doi: 10.1016/j.jgar.2017.04.015

66. Merhi G, Amayri S, Bitar I, Araj GF, Tokajian S. Whole genome-based characterization of multidrug resistant *Enterobacter* and *Klebsiella aerogenes* isolates from Lebanon. *Microbiol Spectr*. (2023) 11:e0291722. doi: 10.1128/spectrum.02917-22

67. Chen Q, Lin Y, Li Z, Lu L, Li P, Wang K, et al. Characterization of a new transposon, *Tn*6696, on a *bla*NDM-1-carrying plasmid from multidrug-resistant *Enterobacter cloacae* ssp. *dissolvens* in China. *Front Microbiol*. (2020) 11:525479. doi: 10.3389/fmicb.2020.525479