



# High Levels of Multi Drug Resistant *Escherichia coli* Pathovars in Preharvest Environmental Samples: A Ticking Time Bomb for Fresh Produce Related Disease Outbreak

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The preharvest environment provides a suitable platform for the emergence and dissemination of multidrug resistant pathogenic bacteria. This could be detrimental to public health and may lead to fresh produce related disease outbreaks when transferred to the food web. This study evaluated the occurrence and antibiogram fingerprints of the pathogenic strains of *Escherichia coli* (*E. coli*) recovered from irrigation water and agricultural soil samples in fresh produce farms in the Eastern Cape Province, South Africa. *E. coli* was isolated, pathotyped and screened for antibiogram fingerprints using standard cultural and molecular methods. An antibiogram-based K-means cluster analysis and neighbour-joining dendrogram was used to elucidate the relatedness of the isolates. Of the confirmed isolates, 37% harboured at least one of the screened virulence genes (VGs) that encode for intestinal and extraintestinal *E. coli* pathovars. The most prevalent pathovar/associated VG in all the samples was Enterohemorrhagic *E. coli*/stx2. Of all the antimicrobials tested, *E. coli* pathovars conferred the highest resistance against ampicillin with a median single antimicrobial resistance (AMR) index of 0.23 (IQR: 0.10–0.38, 95% CI: 0.16–0.27) for pathovars from irrigation water and 0.01 (IQR: 0.01–0.03, 95% CI: 0.01–0.03) for pathovars from agricultural soil. About 60 and 20% of the pathovars from irrigation water and agricultural soil were multi drug resistant with a median multiple AMR index of 0.41 (IQR: 0.25–0.52, 95% CI: 0.31–0.47) and 0.38 (IQR: 0.23–0.66, 95% CI: 0.19–0.69) respectively. The most prevalent resistance genes detected were *sulI*, *catII*, *tetA* and *AmpC* across all sample types. The *bla*<sub>TEM</sub> was the most prevalent extended-spectrum  $\beta$ -lactamase resistance gene detected. A positive correlation between the phenotypic and genotypic AMR profiles of the pathovars in irrigation water samples,  $r = 0.6$ ,  $p \leq 0.01$  and agricultural soil samples,  $r = 0.8$ ,  $p \leq 0.01$  was recorded. The antibiogram based dendrogram generated 11 and 7 clades from irrigation water and agricultural soil samples respectively, indicating that the isolates are highly diversified. The findings revealed the occurrence of highly diversified multidrug resistant *E. coli* pathovars in the preharvest environment, indicating potential public health threats.

**Keywords:** environment, antibiotic resistance, public health, disease outbreak, microbial disease

## INTRODUCTION

Antimicrobial resistance (AMR) is a multifaceted global health problem that increases hospitalization time, treatment costs, morbidity and mortality. Its containment requires a multi-pronged approach which involves the use of the One Health concept. This concept indicates the interrelatedness between the human, animal, food and environmental health spheres, and fosters collaborative and multisectoral efforts to tackle the challenge (Taneja and Sharma, 2019). Sadly, AMR engendered from the environmental sphere has received less attention compared to that from the human and animal health spheres despite the significant variation in the geographical spread of AMR contributed by the environment (O'Neill, 2015).

The environment serves as a key reservoir of antimicrobial resistance genes (ARGs) which are transferable to clinically significant pathogenic bacteria. In terms of the uptake of the novel resistance determinants, the varying ecological niches of the environment provides a highly diversified gene pool that is unmatched to that of the human and animal spheres (Schulz et al., 2017). Of all the environmental niches, the agricultural ecosystem is more complex considering the various activities that influence the tenacity and spread of AMR within the system (Mafiz et al., 2018). The release of wastewater treatment plant (WWTP) effluents to surface water bodies typically used for recreational, domestic and irrigation purposes; the application of bio-solids and organic manure to agricultural soil; animal intrusion on farmlands and water bodies; and the use of reclaimed wastewater for irrigation purposes have led to the expansion of AMR within and beyond the agroecosystem (Iwu et al., 2020). Because fresh produce is almost always in contact with irrigation water and agricultural soil during the preharvest agricultural production, these environmental niches, therefore, serve as key transmission pathways for ARB and their determinants to the food web.

In the past decade, the consumption of fresh produce has been recognized as a potential exposure route of not only foodborne pathogens but also ARB (Pesavento et al., 2014; Araújo et al., 2017). Foodborne diseases caused by pathogenic bacteria in field-grown produce have tremendously increased, leading to significant public health burden and multiple disease outbreaks (CDC, 2014; EFSA, 2017). Pathogenic *E. coli* strains have been implicated in several fresh produce related disease outbreaks, some of which were traced back to the preharvest environment. In 2005, an outbreak in Sweden with about 135 cases was caused by *E. coli* O157:H7 in iceberg lettuce and was traced back to the irrigation water used during the primary production (Söderström et al., 2008). In 2013, another outbreak was caused by enterohaemorrhagic *E. coli* (EHEC) in a fresh salad and the irrigation water used during the primary production was identified as the plausible source of the outbreak (Edelstein et al., 2014).

*E. coli* is a Gram-negative bacterium and a commensal in the human microbiota. Its genomic plasticity has enabled the evolution of the pathogenic strains that can cause human and

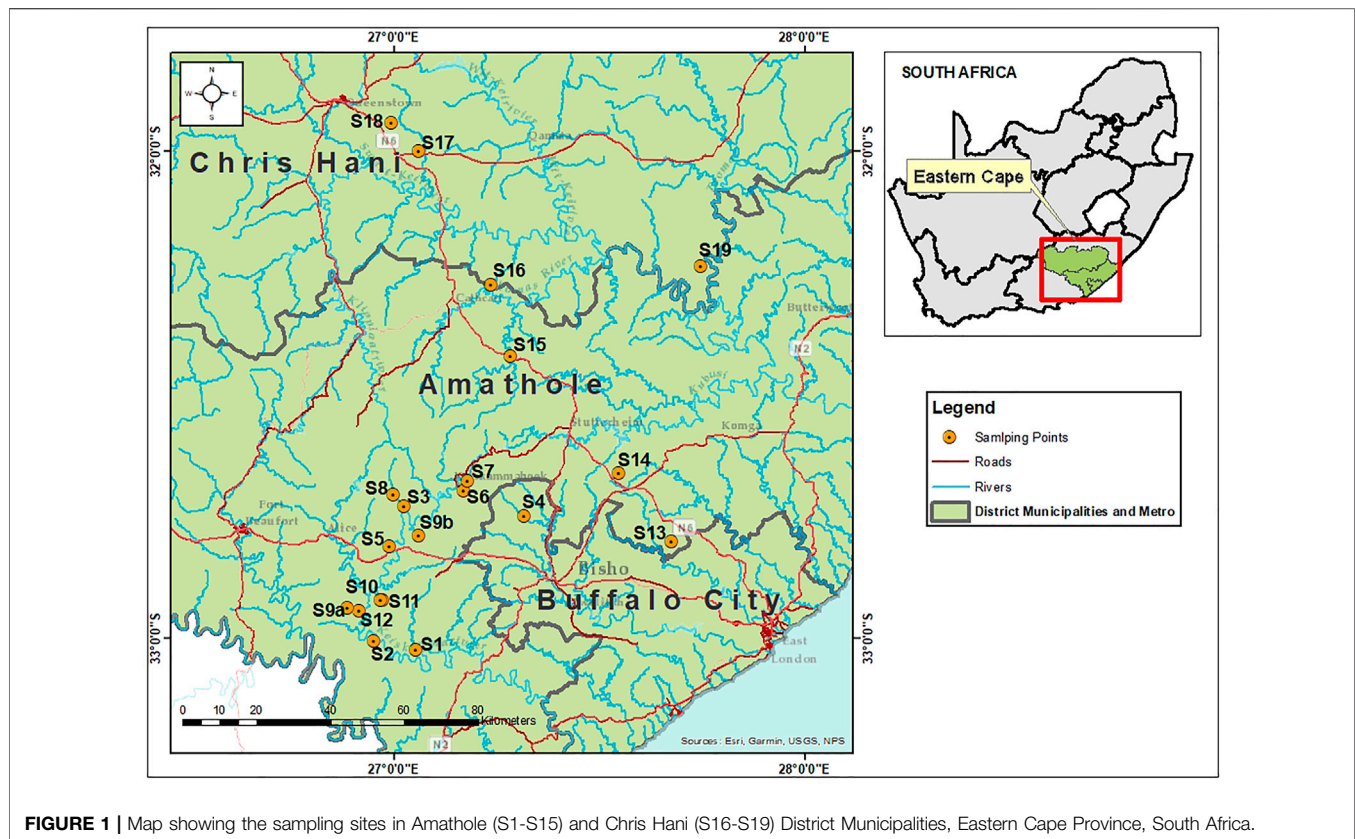
animal diseases (Rojas-Lopez et al., 2018). The pathogenicity of *E. coli* is associated with specific virulence factors including cytotoxic necrotic factors, toxins, effacement factors, adhesins, capsules, haemolysins and invasins. These virulence factors alone are insufficient in determining the pathogenicity of *E. coli*, but targeting them is ideal in characterizing the virulence potential of a given *E. coli* isolate (Kuhnert et al., 2000). The pathogenic strains of *E. coli* are divided into two main groups depending on the location of the disease: intestinal pathogenic *E. coli* (InPEC) and extraintestinal pathogenic *E. coli* (ExPEC). The InPEC strains are transmitted through the faecal-oral route and are associated with diarrheal disease. They are sub-divided into six well-known pathotypes: enterotoxigenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC) and enteropathogenic *E. coli* (EPEC). The ExPEC strains are mainly associated with diseases outside of the intestinal tract. They are subdivided into the uropathogenic *E. coli* (UPEC) which is associated with urinary tract infections and neonatal meningitis *E. coli* (NMEC) which is associated with neonatal meningitis (Rojas-Lopez et al., 2018). Since *E. coli* is found in diverse hosts and environments and can easily acquire resistance, it is well suited for AMR surveillance (Erb et al., 2007).

There is a paucity of data regarding the occurrence of multidrug resistant pathogenic strains of *E. coli* in the preharvest environment in South Africa, despite its significance in the transmission of pathogens to the food web. This study was, therefore, carried out to evaluate the prevalence and diversities of multidrug resistant pathogenic strains of *E. coli* in irrigation water and agricultural soil in fresh produce farms so that appropriate measures can be taken to prevent their transmission to the food web.

## METHODS

### Study Design and Setting

This study involved a cross-sectional laboratory-based analysis of preharvest environmental samples including irrigation water and agricultural soil collected from fresh produce farms in Amathole and Chris Hani District Municipalities, Eastern Cape Province. Eastern Cape Province is the second largest province in South Africa with an area of about 170,000 km<sup>2</sup> and 6 676 590 people according to the 2021 Mid-year population estimates (Statssa, 2021). Amathole District Municipality is located in the central part of the province while Chris Hani District Municipality is located in the north-eastern part of the province. The agricultural and agro-processing sectors in the province are deeply rooted in the Provincial Growth and Development Program (PGDP) (EC Provincial Website, 2020). Fifteen sampling sites (S1-S15) were in Amathole District Municipality, while 4 sampling sites (S16-S19) were in Chris Hani District Municipality. This makes a total of 19 sampling sites as shown in **Figure 1**. These sampling sites were selected after reconnaissance visits were done and permits were granted by the farm owners. The anthropogenic activities identified in the irrigation water sources were animal



**FIGURE 1** | Map showing the sampling sites in Amathole (S1-S15) and Chris Hani (S16-S19) District Municipalities, Eastern Cape Province, South Africa.

intrusions, swimming, discharge of poultry and swine effluents and dumping of refuse while the anthropogenic activities identified on the farmlands were animal intrusion and soil amendment with organic and inorganic fertilizers as detailed in **Supplementary Table S1**.

### Sample Collection

A grab sampling technique was employed in this study. Nineteen irrigation water samples (1 L each) and 13 agricultural soil samples (30 g each) were collected from the sampling sites using sterile 1L sampling bottles and sterile plastic bags respectively. The farms in S1, S4, S6, S10, S15, and S19 were inaccessible. As such, agricultural soil samples were not collected and only irrigation water samples were collected. All the samples were collected in triplicates, stored in a cooler box with ice and shipped to the laboratory within 4 h for microbiological analysis.

### Isolation of Presumptive *E. coli*

In the laboratory, 10 ml of the irrigation water samples and 10 g of the agricultural soil samples were transferred to 90 ml of trypticase Soy Broth (TSB) (Laboratorios Conda, Pronadisa, South Africa) for the enrichment of *E. coli* in the samples. The broth was incubated at 37°C for 24 h after which a loop full was streaked on *E. coli* chromogenic agar (ECA) (Laboratorios Conda, Pronadisa, South Africa). After 24 h of incubation at 37°C, blue colonies on ECA were further purified on Eosin

Methylene Blue (EMB) Agar (Laboratorios Conda, Pronadisa, South Africa) and incubated at 37°C for 24 h. At least 10 morphologically clear and distinct isolates from each sample with green metallic sheen were randomly picked as presumptive *E. coli* and stored in 25% glycerol stock at -80°C for further analysis.

### Molecular Confirmation of the Presumptive *E. coli* Isolates and the Characterization of the Pathogenic Strains

The presumptive *E. coli* isolates were confirmed by targeting the housekeeping 4-methyl umbelliferyl-glucuronide (*uidA*) gene marker in a polymerase chain reaction (PCR) assay as described by Nontongana et al. (2014). Before this, the isolates were resuscitated using nutrient broth (Merck, SA) and the DNA of the isolates were extracted using the boiling technique as described by Maugeri et al. (2004) and López-Saucedo et al. (2003). The confirmed *E. coli* isolates were delineated into 6 intestinal pathogenic strains (EAEC, EIEC, DAEC, ETEC, EPEC, EHEC) and 2 extraintestinal pathogenic strains (NMEC, UPEC) as described by Titilawo et al. (2015b) in simplex PCR assays. The housekeeping gene, virulence genes, primer sequences, amplicon sizes and cycling conditions used to confirm the presumptive *E. coli* isolates and characterize them into their pathotypes are shown in **Table 1**. All the PCR assays were carried out using the MyCycler™ thermal Cycler

**TABLE 1** | Primer sequence, PCR cycling conditions and expected amplicon sizes for the confirmation and delineation of *E. coli* into its pathotypes.

Target strains	Gene	Primer sequence (5-3')	PCR cycling conditions	Amplicon size	Reference
<i>E. coli</i>	<i>uidA</i>	F: AAAACGGCAAGAAAAAGCAG R: ACGCGTGGTTAACAGTCT TGCG	94°C, 5 min; 35[95°C, 30 s; 58°C, 1 min; 72°C, 1 min] 72°C, 8 min	147	Moyo et al. (2007)
Intestinal pathogenic <i>E. coli</i>					
EAEC	<i>eagg</i> adhesion gene	F: AGACTCTGGCGAAAGACT GTATC R: ATGGCTGTCTGTAATAGATGA GAAC	95°C, 15 min; 35 cycles [94°C, 45 s; 55°C, 45 s; 68°C, 2 min]; 72°C, 5 min	194	Kong et al. (2002)
EIEC	<i>ipaH</i> invasion gene	F: CTC GGCAAGTTTTAATAG TCTGG R: GTGGAGAGCTGAAGTTTC TCTGC	95°C, 5 min; 30 cycles [95°C, 45 s; 55°C, 45 s; 72°C, 45 s]; 72°C, 10 min	320	Vidal et al. (2005)
DAEC	<i>daaE</i> adhesion gene	F: GAACGTTGGTTAATGTGG GGTAA R: TATTACCCGGTCGTTATCAGT	94°C, 2 min; 40 cycles [92°C, 30 s; 59°C, 30 s; 72°C, 30 s]; 72°C, 5 min	542	Vidal et al. (2005)
ETEC	<i>lt</i> toxin gene	F: GGCGACAGATTATACCGTGC R: CGGTCTCTATATTCCCTGTT	94°C, 2 min; 35 cycles [94°C, 1 min; 55°C, 1 min, 72°C, 1 min]; 72°C, 5 min	450	López-Saucedo et al. (2003)
EPEC	<i>eae</i> adhesion gene	F: TCAATGCAGTTCGGTTATCAGTT R: GTAAAGTCCGTTACCCCA ACCTG	95°C, 15 min; 35 cycles [94°C, 45 s; 55°C, 45 s; 68°C, 2 min]; 72°C, 5 min	482	Stacy-Phipps et al. (1995)
	<i>bfp</i> adhesion gene	F: AGACTCTGGCGAAAAGTGTATC R: ATGGCTGTCTGTAATAGATGA GAAC	94°C, 2 min; 40 cycles [94°C, 1 min; 55°C, 1 min; 72°C, 1 min]; 72°C, 5 min	300	Stacy-Phipps et al. (1995)
EHEC	<i>stx1</i> toxin gene	F: ATAAATGCCATTCTGTTG ACTAC R: AGAACGCCCACTGAGATCATC	94°C, 3 min; 35 cycles [93°C, 60 s; 55°C, 60 s; 72°C, 60 s]; 72°C, 7 min	180	Paton and Paton, (1998)
	<i>stx2</i> toxin gene	F: GGCACGTCTGAAAAGTGTCC R: TCGCCAGTTATCTGACATTCTG	94°C, 3 min; 35 cycles [93°C, 60 s; 55°C, 60 s; 72°C, 60 s]; 72°C, 7 min	255	Paton and Paton, (1998)
Extraintestinal pathogenic <i>E. coli</i>					
NMEC	<i>ibeA</i> invasion gene	F: TGGAACCCCGCTCGTAATATAC R: CTGCCTGTTCAAGCATTG CA	94°C, 2min; 30 cycles [94°C, 1 min; 55°C, 1 min; 72°C, 1 min]; 72°C for 5 min	342	Wijetunge et al. (2015)
UPEC	<i>papC</i> adhesion gene	F: GACGGCTGTACTGCAGGGTGT GGCG R: TATCCTTTCTGCAGGGAT GCAATA	94°C, 2 min; 30 cycles [94°C, 1 min; 55°C, 1 min; 72°C, 1 min] 72°C for 5 min	328	Dadi et al. (2020)

PCR system (BioRad, United States) while the electrophoresed gels were visualized under the ultraviolet trans-illuminator (Alliance 4.7).

## Antimicrobial Susceptibility Testing and the Single Antimicrobial Resistance Indexing of the Pathogenic *E. coli* Strains

The disk diffusion test was used to evaluate the AS patterns of the pathogenic strains of *E. coli* following the recommendation of the Clinical Laboratory Standards Institute (CLSI, 2018). Confirmed *E. coli* pathotypes were picked from an overnight pure culture on nutrient agar, emulsified in sterile normal saline and adjusted to match a 0.5 McFarland standard. The mixture was evenly inoculated on Mueller-Hinton agar (Merck, South Africa) using sterile swabs. The test antibiotics were then placed on the agar using a disc dispenser. This was then incubated at 37°C for 24 h. All the zone of inhibition were measured in millimeters (mm) and the values were interpreted using the CLSI recommended

standards (CLSI, 2018). The outcomes of the susceptibility tests were categorized as either Resistant (R), Intermediate (I) or Susceptible (S) to the test antibiotics. A total of 16 antibiotics belonging to 10 classes that are commonly used to treat infections caused by pathogenic *E. coli* were used to carry out the AS testing. They include; tetracyclines [doxycycline (DXT/30 µg), tetracycline (T/30 µg)], carbapenems [meropenem (MEM/10 µg), imipenem (IMI/10 µg)], fluoroquinolones [norfloxacin (NOR/30 µg), ciprofloxacin (CIP/5 µg)], phenicols [chloramphenicol (C/30 µg)], quinolones [nalidixic acid (NA/30 µg)], cepheims [cefuroxime (CXM/30 µg), cefotaxime (CTX/30 µg)], β-lactams [ampicillin (AP/10 µg), amoxicillin-clavulanic acid (AUG/30 µg)], sulfonamides [trimethoprim/sulphamethoxazole (TS/25 µg:25 µg)], aminoglycosides [amikacin (AK/30 µg), gentamycin (GM/10 µg)] and nitrofurans [nitrofurantoin (NI/300 µg)].

The Single Antimicrobial Resistance Index (SARI) of the pathogenic strains of *E. coli* retrieved from the preharvest environmental samples were evaluated using Eq. 1. The SARI



provides values that range from 0 to 1. Isolates with SARI close to 1 are highly resistant against the test antimicrobials (Hernandez et al., 2021).

$$\text{SARI} = \frac{R + I}{R + I + S}$$

R, I and S represent the number of isolates with resistant, intermediate and susceptible outcomes respectively.

## Multiple Antibiotic Resistance Phenotyping and Indexing of the *E. coli* Pathovars

*E. coli* pathovars that were non-susceptible to one or more antibiotics in three or more antimicrobial categories were considered multidrug resistant (MDR) (Magiorakos et al., 2012).

The multiple antimicrobial resistance phenotypes (MARPs) of the MDR isolates were evaluated as described by Titilawo et al. (2015c), while their multiple antimicrobial resistance indices (MARI) were evaluated using Eq. 2 (Krumperman, 1983).

$$\text{MARI} = \frac{\text{Number of antibiotics a bacteria is resistant against}}{\text{Total number of test antibiotics}}$$

MARI  $\geq 0.2$  indicate that the pathogen is from a “high-risk environment” where antimicrobials are indiscriminately used (Krumperman, 1983).

## Molecular Screening of Antimicrobial Resistance Genes in Antibiotic Resistant *E. coli* Pathovars

Phenotypic antimicrobial resistant of *E. coli* pathovars were screened for putative antimicrobial resistance genes (ARGs) using PCR assays. The simplex, duplex and multiplex PCR assays were used to screen for 19 ARGs encoding tetracycline (*tetA*, *tetB*, *tetC*, *tetD*, *tetE*, *tetG*, *tetK*, *tetL*, *tetM*), sulfonamide (*sulI*, *sulII*), phenicol (*cmIA1*, *catI*, *catII*) and aminoglycoside [*strA*, *aadA*, *aac(3)-IIa(aacC2)*, *aph(3)-Ia(aphA1)*, *aph(3)-IIa(aphA2)*] resistance as described by (Titilawo et al., 2015a). **Supplementary Table S2** details the genomic sequences of these genes, amplicon sizes, and the PCR cycling conditions. Additionally, 20 ARGs encoding for *AmpC*  $\beta$ -lactamase, ESBLs including *bla*<sub>TEM</sub> (TEM-1 and TEM-2), *bla*<sub>SHV</sub> (SHV-1), *bla*<sub>OXA-1-like</sub> (OXA-1, OXA-4, OXA-30), *bla*<sub>OXA-48-like</sub> (OXA-48), *bla*<sub>CTX-M-1</sub> (CTX-M-1, CTX-M-3, CTX-M-15), *bla*<sub>CTX-M-2</sub> (CTX-M-2), *bla*<sub>CTX-M-9</sub> (CTX-M-9, CTX-M-14), *bla*<sub>CTX-M group 8/25</sub> (CTX-M-8, CTX-M-25, CTX-M-26, CTX-M-39 to CTX-M-41), *bla*<sub>VEB</sub> (VEB-1 to VEB-6), *bla*<sub>PER</sub> (PER-1, PER-3), *bla*<sub>GES</sub> (GES-1 to 8, GES-11), plasmid-mediated *AmpC* including *bla*<sub>ACC</sub> (ACC-1, ACC-2), *bla*<sub>FOX</sub> (FOX-1 to FOX-5), *bla*<sub>MOX</sub> (MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11, CMY-19), *bla*<sub>DHA</sub> (DHA-1, DHA-2), *bla*<sub>CIT</sub> (LAT-1 to LAT-3, BIL-1, CMY-2 to MY-7, CMY-12 to CMY-18 and CMY-21 to CMY-23), *bla*<sub>EBC</sub> (ACT-1, MIR-1) and carbapenemases including *bla*<sub>IMP</sub> (IMP variants

except for IMP-9, IMP-16, IMP-18, IMP-22 and IMP-25), *bla*<sub>VIM</sub> (VIM-1, VIM-2), *bla*<sub>KPC</sub> (KPC-1 to KPC-5) were screened for in a simplex and multiplex PCR assays as described by Dallenne et al. (2010). The genomic sequences of these genes, amplicon sizes, and PCR cycling conditions are detailed in **Supplementary Table S3**.

## Assessment of the Multiple Antibiotic Resistance Genotypes in Pathogenic *E. coli* Strains

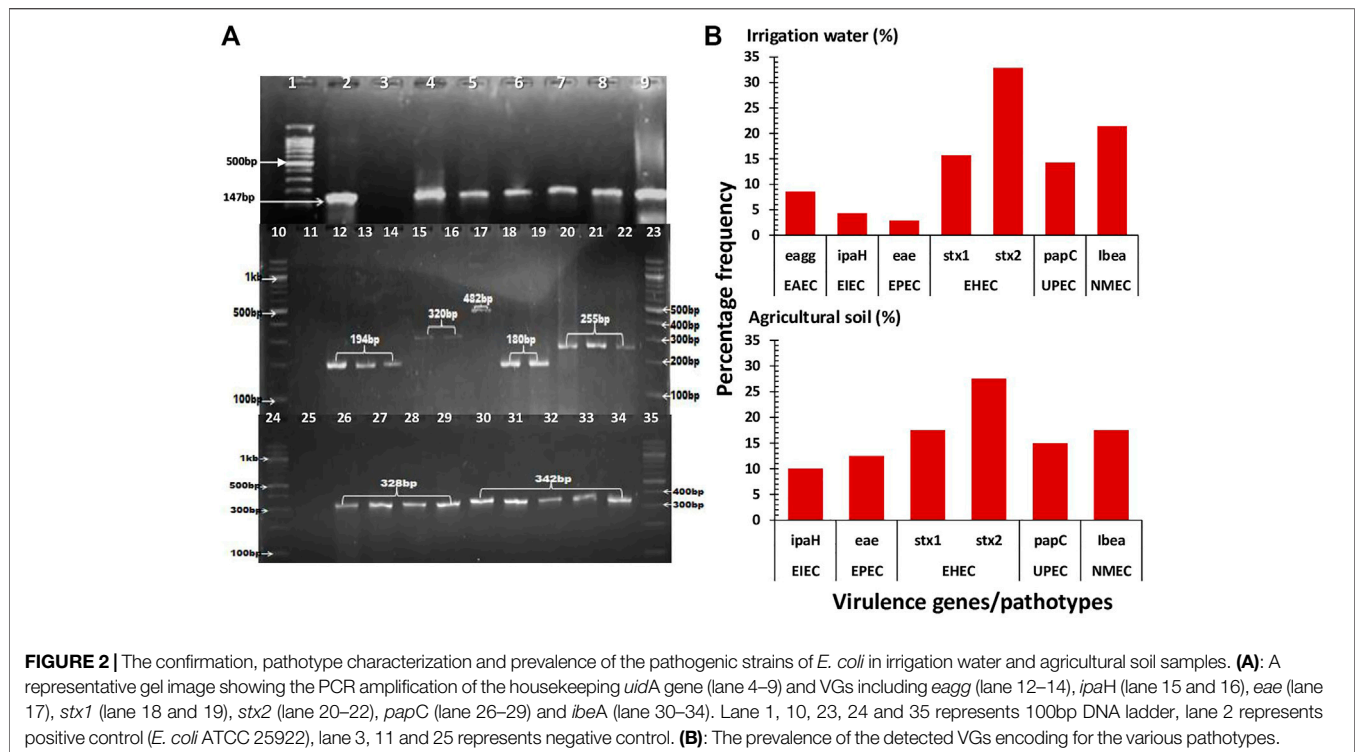
The multiple antimicrobial resistance genotypes (MARGs) of the pathogenic *E. coli* strains harbouring  $\geq 2$  ARGs in each sample type were evaluated. The patterns of the MARGs were elucidated as described by Iwu and Okoh (2020).

## Evaluation of the Correlation Between the Phenotypic and Genotypic Characteristics of the *E. coli* Pathovars and Their Antibiogram-Based Diversities

The correlation between the phenotypic and genotypic AMR characteristics of the pathogenic strains of *E. coli* retrieved from irrigation water and agricultural soil was evaluated using appropriate statistical analysis. A heatmap was constructed to display the phenotypic and genotypic AMR characteristics of all the pathogenic strains of *E. coli* retrieved from irrigation water and agricultural soil samples. Based on the antibiogram fingerprints of the isolates, a cluster analysis was done using the k-means clustering technique to elucidate the relatedness of the isolates. This was portrayed by a neighbour-joining dendrogram using a Jaccard similarity index and the generated clades.

## Statistical Analysis

Data obtained from this study were captured in Microsoft Excel (version 2016) and subjected to descriptive and inferential statistical analysis using STATA 15 (Stata Corp LLC 4905, Lakeway Drive College Station, Texas 77,845, United States). The data were analysed for normality using the Shapiro-Wilk test, which rejects the null hypothesis of normality when  $p < 0.05$  (Ahad et al., 2011). Normally distributed continuous variables were described using means, standard deviation (SD) and 95% confidence interval (CI), otherwise described using median, interquartile range (IQR) and 95% confidence interval (CI). Dichotomous and categorical variables were described using percentage proportions. The SARI and MARI of the pathogenic strains of *E. coli* retrieved from irrigation water and agricultural soil samples were compared using an unpaired samples *t*-test. The Spearman's rank correlation test was used to assess the correlation between the phenotypic and genotypic AMR characteristics of the pathogenic strains of *E. coli* retrieved from the preharvest environmental samples. A two-tailed *p*-value of 0.05 was set to be statistically significant. The antibiogram-based K-means cluster analysis and neighbour-joining dendrogram was carried out using the



Paleontological statistics software (version 3.14, Oslo, Norway).

## RESULTS

### Confirmation and Pathogenic Characterization of *E. coli* Isolates Retrieved From the Preharvest Environmental Samples

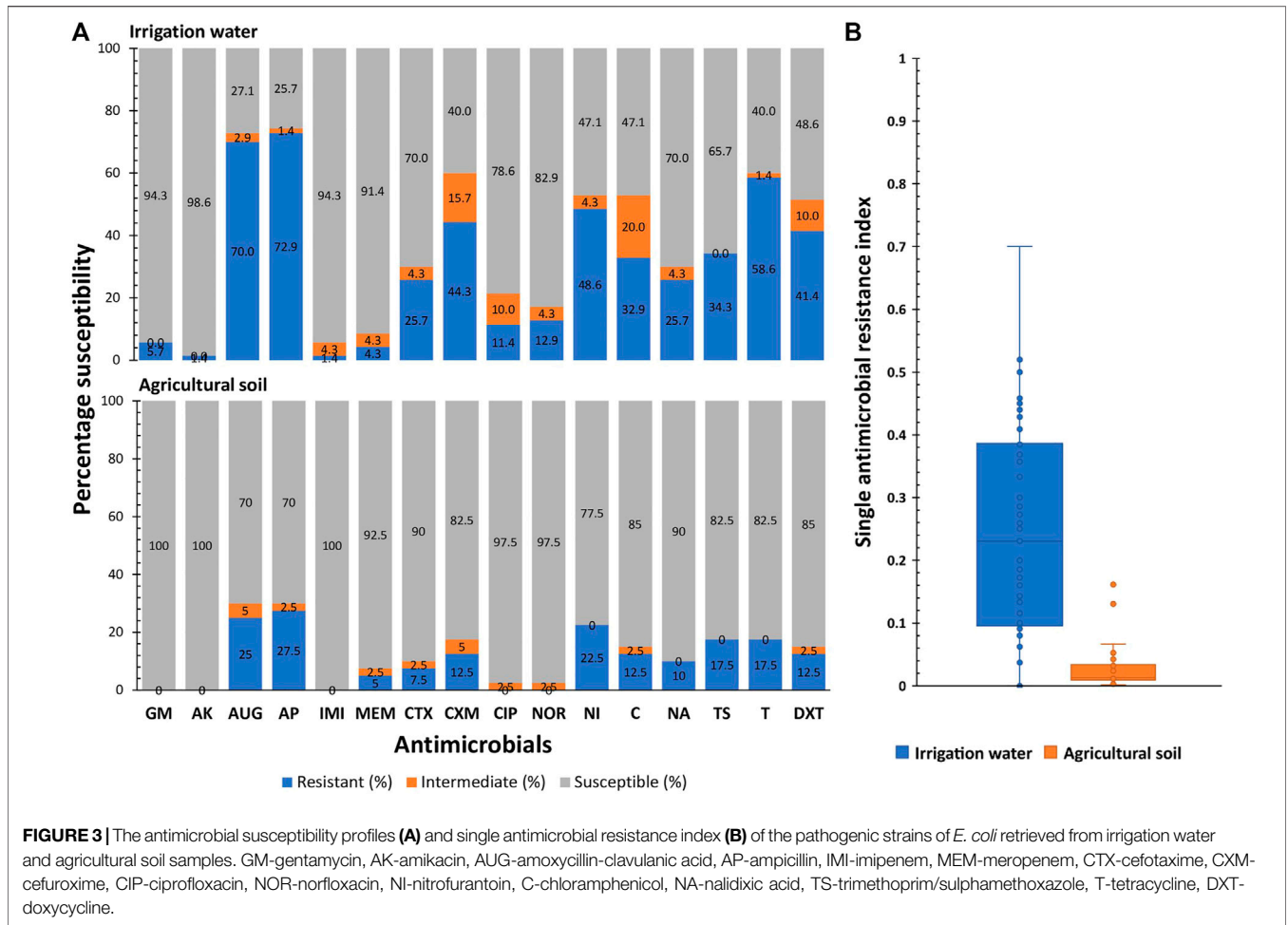
A total of 350 presumptive *E. coli* isolates were retrieved, of which 200 were from irrigation water samples and 150 were from agricultural soil samples. Of this, 300 (86%) were confirmed to be *E. coli*. Specifically, 180 (60%) confirmed isolates were retrieved from irrigation water samples while 120 (40%) confirmed isolates were retrieved from agricultural soil samples. Out of the confirmed *E. coli* isolates ( $n = 300$ ), 110 (37%) were found to be pathogenic harbouring at least one of the screened virulence genes. Of this, 70 (64%) pathogenic strains were retrieved from irrigation water samples while 40 (36%) pathogenic strains were retrieved from agricultural soil samples. The PCR amplification of the housekeeping *uidA* gene used in the confirmation of *E. coli* as well as the virulence genes used in the characterization of its pathotypes are shown in **Figure 2A**.

In irrigation water samples, the InPEC pathotypes/encoded VGs detected include EAEC/*eagg*, EIEC/*ipaH*, EPEC/*eae*, EHEC/*stx1* and EHEC/*stx2* while the ExPEC pathotype/encoded VGs detected include UPEC/*papC* and

NMEC/*lbea*. In agricultural soil samples, the InPEC pathotypes/encoded VGs detected include EIEC/*ipaH*, EPEC/*eae*, EHEC/*stx1* and EHEC/*stx2* while the ExPEC pathotypes/encoded VGs detected include UPEC/*papC* and NMEC/*lbea*. The prevalence of the detected pathotypes in irrigation water samples ranged from 2.9% (EPEC/*eae*) to 32.9% (EHEC/*stx2*) while the prevalence of the detected pathotypes in agricultural soil samples ranged from 10% (EIEC/*ipaH*) to 27.5% (EHEC/*stx2*) as shown in **Figure 2B**.

### Antimicrobial Susceptibility Profiles and Single Antimicrobial Resistance Indices (SARI) of the Pathogenic Strains of *E. coli* Isolates in Preharvest Environmental Samples

The antimicrobial susceptibility profiles and SARI of the pathogenic strains of *E. coli* retrieved from irrigation water and agricultural soil samples are shown in **Figure 3**. In irrigation water samples, the percentage susceptibility of the isolates to the test antimicrobials ranged from 25.7% (ampicillin) to 98.6% (amikacin), while the percentage resistance of the isolates against the test antimicrobials ranged from 1.4% (amikacin and imipenem) to 72.9% (ampicillin). High susceptibility rates were also observed for gentamicin (94.3%), imipenem (94.3%) and meropenem (91.4%), and high resistance rates were also observed for amoxicillin-clavulanic acid (70.0%) and tetracycline (58.6%). The SARI of the pathogenic *E. coli* retrieved from irrigation



**FIGURE 3 |** The antimicrobial susceptibility profiles (A) and single antimicrobial resistance index (B) of the pathogenic strains of *E. coli* retrieved from irrigation water and agricultural soil samples. GM-gentamicin, AK-amikacin, AUG-amoxicillin-clavulanic acid, AP-ampicillin, IMI-imipenem, MEM-meropenem, CTX-cefotaxime, CXM-cefuroxime, CIP-ciprofloxacin, NOR-norfloxacin, NI-nitrofurantoin, C-chloramphenicol, NA-nalidixic acid, TS-trimethoprim/sulphamethoxazole, T-tetracycline, DXT-doxycycline.

water samples ranged from 0.0 to 0.70 with a median score of 0.23 (IQR: 0.10 to 0.38, 95% CI: 0.16–0.27).

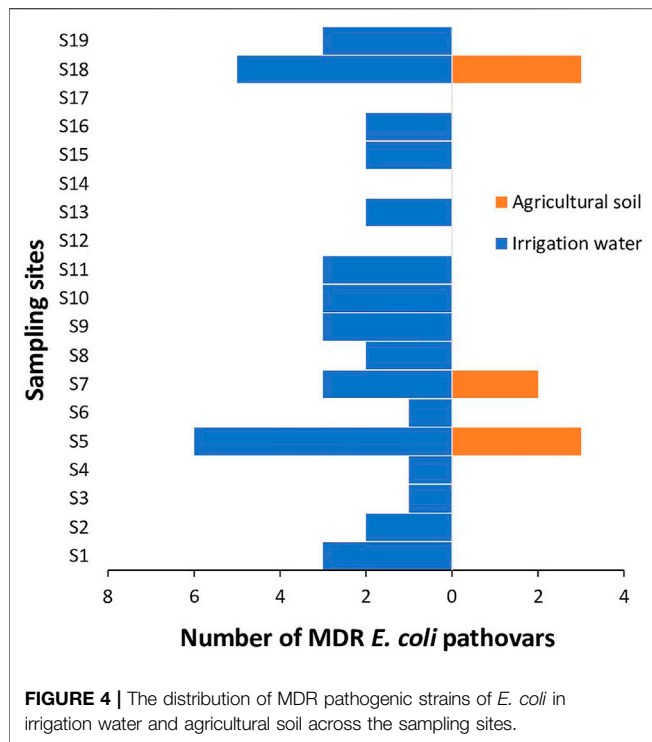
In agricultural soil samples, the percentage susceptibility of the isolates to the test antimicrobials ranged from 70% (amoxicillin-clavulanic acid and ampicillin) to 100% (gentamicin, amikacin and imipenem), while the percentage resistance of the isolates against the test antimicrobials ranged from 0.0% (gentamicin, amikacin and imipenem) to 27.5% (ampicillin). High susceptibility rates were also observed for ciprofloxacin (97.5%), norfloxacin (97.5%), meropenem (92.5%), cefotaxime (90%) and nalidixic acid (90%) and high resistance rates were also observed for amoxicillin-clavulanic acid (25%), nitrofurantoin (22.5%), tetracycline (17.5%) and trimethoprim/sulphamethoxazole (17.5%). The SARI of the pathogenic *E. coli* retrieved from agricultural soil samples ranged from 0.002 to 0.16 with a median score of 0.01 (IQR: 0.01 to 0.03, 95% CI: 0.01–0.03).

The SARI of the antimicrobial resistant pathogenic *E. coli* strains retrieved from irrigation water was significantly higher than that of the pathogenic *E. coli* strains retrieved from agricultural soil;  $t(108) = 7.8, p \leq 0.01$ .

### The Multiple Antimicrobial Resistance Phenotypes and Multiple Antimicrobial Resistance Indices of the Pathogenic Strains of *E. coli* Recovered From the Preharvest Environmental Samples

Of the pathogenic *E. coli* strains ( $n = 70$ ) recovered from the irrigation water samples, 60 (85.7%) were phenotypically resistant against at least one antimicrobial, while 42 (60.0%) were multidrug resistant (MDR). The majority of the MDR *E. coli* pathogens in irrigation water samples were retrieved from S5 and S18 as shown in **Figure 4**. The MARPs and MARI of the MDR isolates are shown in **Table 2**. A total of 38 patterns of MARPS was observed most of which ( $n = 34$ ) appeared uniquely. Four MARPS patterns including “NI-T-DXT”, “AUG-AP-CTX-NI-C”, “AUG-AP-CXM-NI-C-NA-TS-T-DXT”, “GM-AUG-AP-CIP-NOR-C-NA-TS-T-DXT” were observed in duplicates. The MARI of the MDR pathogenic *E. coli* from irrigation water samples ranged from 0.2 to 0.8 with a median score of 0.41 (IQR: 0.25 to 0.52, 95% CI: 0.31–0.47).

Of the pathogenic *E. coli* strains ( $n = 40$ ) recovered from the agricultural soil samples, 15 (37.5%) were phenotypically



resistant against at least one antimicrobial, while 8 (20.0%) were (MDR). The MDR *E. coli* pathovars in agricultural soil samples were retrieved from S5, S7 and S18 as shown in Figure 4. The MARPs and MARI of the MDR isolates are shown in Table 3. A total of 6 patterns of MARPs was observed most of which ( $n = 4$ ) appeared uniquely. Two MARPs patterns including “AUG-AP-NI-TS-T-DXT” and “AUG-AP-MEM-CTX-CXM-NI-C-NA-TS-T-DXT” were observed in duplicates. The MARI of the MDR pathogenic *E. coli* from agricultural soil samples ranged from 0.2 to 0.7 with a median score of 0.38 (IQR: 0.23 to 0.66, 95% CI: 0.19–0.69).

There was no significant difference between the MARI of the MDR pathogenic *E. coli* strains retrieved from irrigation water and that from agricultural soil;  $t(56) = 0.3, p = 0.7$ .

### Detection and Prevalence of Antimicrobial Resistance Genes in Phenotypically Resistant Pathogenic Strains of *E. coli*

The ARGs detected in phenotypically resistant pathogenic strains of *E. coli* recovered from irrigation water samples include *catII* phenicol resistance encoding gene, *tetA* and *tetB* tetracycline resistance-encoding genes, *sulI* and *sulII* sulfonamide resistance-encoding genes, *AmpC*  $\beta$ -lactam resistance-encoding gene and *bla*<sub>TEM</sub>, *bla*<sub>PER</sub>, and *bla*<sub>GES</sub> ESBLs. The frequency of occurrence of these genes ranged from 4.2% for *bla*<sub>PER</sub> and *bla*<sub>GES</sub> to 75.0% for *sulI* as shown in Figure 5.

In phenotypically resistant pathogenic strains of *E. coli* recovered from agricultural soil, the ARGs detected include *catII* phenicol resistance encoding gene, *tetA* and *tetB*

tetracycline resistance-encoding genes, *sulII* sulfonamide resistance-encoding genes, *AmpC*  $\beta$ -lactam resistance-encoding gene and *bla*<sub>TEM</sub> ESBL. The frequency of occurrence of these genes ranged from 10.0% for *bla*<sub>TEM</sub> to 80.0% for *catII* and *AmpC* as shown in Figure 5.

### The Multiple Antimicrobial Resistance Genotypes in the pathogenic Strains of *E. coli* Recovered From Preharvest Environmental Samples

In irrigation water samples, 26 (43.3%) of the phenotypically resistant pathogenic strains of *E. coli* ( $n = 60$ ) harboured multiple ( $\geq 2$ ) ARGs, some of which were a combination of non- $\beta$ -lactams/ESBLs and  $\beta$ -lactams/ESBLs. The MARGs of these isolates were mapped as shown in Table 4 and a total of 18 patterns were observed, most of which occurred uniquely. Four of the patterns were observed in duplicates while two of the patterns were observed in triplicates.

In agricultural soil samples, 5 (33.3%) of the phenotypically resistant pathogenic strains of *E. coli* ( $n = 15$ ) harboured multiple ( $\geq 2$ ) ARGs, some of which were a combination of non- $\beta$ -lactams/ESBLs and  $\beta$ -lactams/ESBLs. The MARGs of these isolates were mapped as shown in Table 5 and a total of five patterns were observed, all of which occurred uniquely.

### The Correlation Between the Phenotypic and Genotypic Characteristics of *E. coli* Pathovars in the Preharvest Environmental Samples and Their Antibigram-Based Diversities

There was a positive correlation between the phenotypic and genotypic AMR profiles of the pathogenic strains of *E. coli* retrieved from irrigation water samples,  $r = 0.6, p \leq 0.01$  and agricultural soil samples,  $r = 0.8, p \leq 0.01$ . Based on the antibiogram fingerprints of the pathogenic strains of *E. coli* retrieved from the pre-harvest environmental samples, 11 clades (A to K) of the isolates from irrigation water samples were identified as shown in Figure 6, while 7 clades (A to G) of the isolates from the agricultural soil samples were identified as shown in Figure 7. Two isolates from the irrigation water samples and only one isolate from the agricultural soil samples did not cluster with other isolates.

## DISCUSSION

The occurrence of antimicrobial resistant *E. coli* in the environment has been proposed to indicate the presence of other public health and environmentally significant antimicrobial resistant bacteria and clinically relevant ARGs (EFSA, 2008; Gekenidis et al., 2018). In this study, we evaluated the prevalence and diversities of multidrug resistant pathogenic strains of *E. coli* in preharvest environmental samples



**TABLE 2 |** The MARPs and MARI of the pathogenic strains of *E. coli* retrieved from irrigation water samples.

SN	MAR Phenotypes	No. of antibiotics	No. of isolates	MARI
1	AUG-AP-TS	3	1	0.2
2	AP-T-DXT	3	1	0.2
3	NI-T-DXT	3	2	0.2
4	AUG-AP-T	3	1	0.2
5	AUG-AP-T-DXT	4	1	0.3
6	AUG-AP-C-T	4	1	0.3
7	NOR-NI-T-DXT	4	1	0.3
8	AUG-NI-T-DXT	4	1	0.3
9	AUG-AP-CXM-T	4	1	0.3
10	AUG-AP-TS-T-DXT	5	1	0.3
11	AUG-AP-C-T-DXT	5	1	0.3
12	AUG-AP-CXM-NI-T	5	1	0.3
13	AUG-AP-CTX-NI-C	5	2	0.3
14	AUG-AP-CTX-CXM-NI	5	1	0.3
15	AUG-AP-NI-T-DXT	5	1	0.3
16	AUG-AP-CTX-CXM-NI-T	6	1	0.4
17	AP-CXM-NI-TS-T-DXT	6	1	0.4
18	AP-CTX-CXM-NI-T-DXT	6	1	0.4
19	AUG-AP-CXM-NI-C-T	6	1	0.4
20	AUG-AP-NI-C-TS-T-DXT	7	1	0.4
21	AUG-AP-C-NA-TS-T-DXT	7	1	0.4
22	AUG-AP-CXM-NI-C-NA-TS	7	1	0.4
23	AUG-AP-CTX-CXM-NI-C-TS	7	1	0.4
24	AUG-AP-CXM-NI-NA-T-DXT	7	1	0.4
25	AUG-AP-CTX-CXM-NI-T-DXT	7	1	0.4
26	AUG-AP-CTX-CXM-NI-NA-TS-T	8	1	0.5
27	AUG-AP-MEM-CTX-CXM-NI-TS-T	8	1	0.5
28	AUG-AP-CXM-NI-NA-TS-T-DXT	8	1	0.5
29	AUG-AP-CTX-CXM-NI-TS-T-DXT	8	1	0.5
30	AUG-AP-CXM-NI-C-NA-TS-T-DXT	9	2	0.6
31	AUG-AP-CTX-CXM-CIP-NA-TS-T-DXT	9	1	0.6
32	AUG-AP-CTX-CXM-NI-C-NA-TS-T-DXT	10	1	0.6
33	GM-AUG-AP-CIP-NOR-C-NA-TS-T-DXT	10	2	0.6
34	AUG-AP-CIP-NOR-NI-C-NA-TS-T-DXT	10	1	0.6
35	AUG-AP-CTX-CXM-CIP-NOR-NA-TS-T-DXT	10	1	0.6
36	AUG-AP-CXM-CIP-NOR-NI-C-NA-TS-T-DXT	11	1	0.7
37	GM-AK-AUG-AP-IMI-MEM-CTX-CXM-NI-C-TS-T	12	1	0.8
38	GM-AUG-AP-CTX-CXM-CIP-NOR-NI-C-NA-TS-T	12	1	0.8

SN, serial number; GM-gentamycin, AK-amikacin, AUG-amoxicillin clavulanic acid, AP-ampicillin, IMI-imipenem, MEM-meropenem, CTX-cefotaxime, CXM-cefuroxime, CIP-ciprofloxacin, NOR-norfloxacin, NI-nitrofurantoin, C-chloramphenicol, NA-nalidixic acid, TS-trimethoprim/sulphamethoxazole, T-tetracycline, DXT-doxycycline.

**TABLE 3 |** The MARPs and MARI of the pathogenic strains of *E. coli* retrieved from agricultural soil samples.

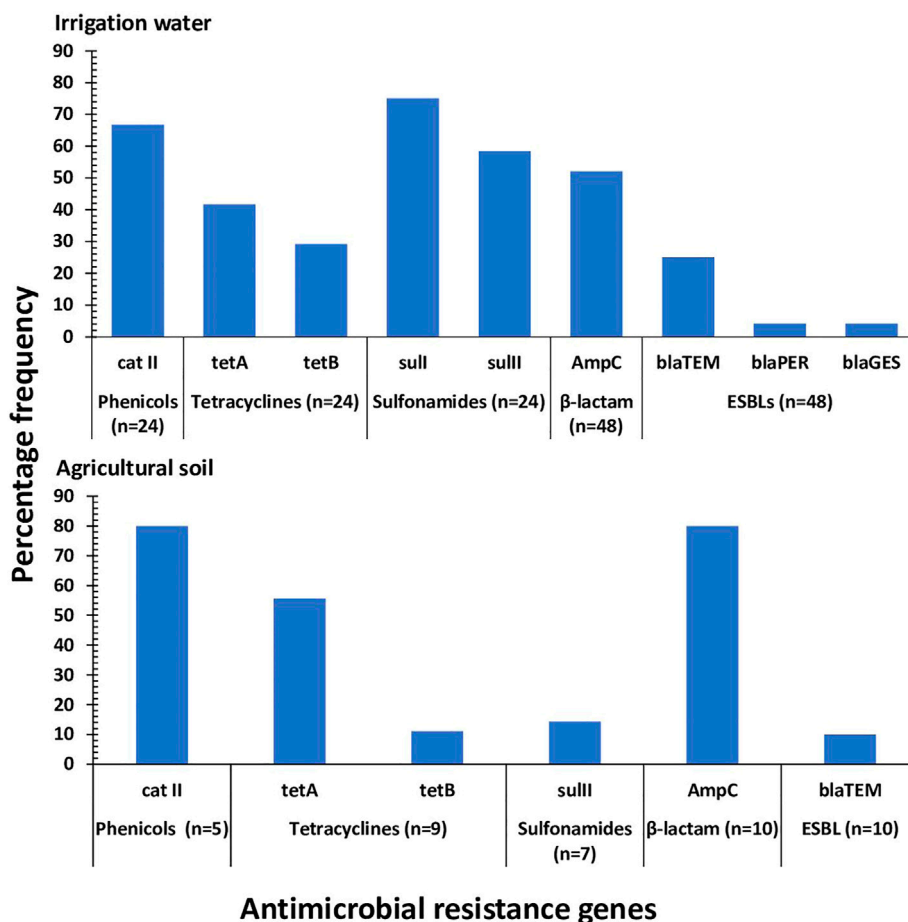
SN	MAR Phenotypes	No. of antibiotics	No. of isolates	MARI
1	AUG-AP-CXM	3	1	0.2
2	AP-TS-T	3	1	0.2
3	AUG-AP-NI-TS-T-DXT	6	2	0.4
4	AUG-AP-CXM-NI-NA-TS	6	1	0.4
5	AUG-AP-CTX-CXM-NI-C-NA-TS-T	9	1	0.6
6	AUG-AP-MEM-CTX-CXM-NI-C-NA-TS-T-DXT	11	2	0.7

SN, serial number; GM-gentamycin, AK-amikacin, AUG-amoxicillin clavulanic acid, AP-ampicillin, IMI-imipenem, MEM-meropenem, CTX-cefotaxime, CXM-cefuroxime, CIP-ciprofloxacin, NOR-norfloxacin, NI-nitrofurantoin, C-chloramphenicol, NA-nalidixic acid, TS-trimethoprim/sulphamethoxazole, T-tetracycline, DXT-doxycycline.

including irrigation water and agricultural soil so that appropriate measures can be taken to minimize their occurrence and transmission to the food web.

A high proportion of the presumptive isolates in both irrigation water and agricultural soil in this study were confirmed to be *E. coli*, many of which were pathogenic

strains harbouring at least one of the screened virulence genes. Four InPEC including EAEC, EIEC, EPEC, EHEC and 2 ExPEC including UPEC and NMEC were detected in the irrigation water samples in this study. Similar pathotypes were detected in a surface water body which may be used for irrigation purposes in Australia (Sidhu et al., 2013). Three InPEC including EIEC,



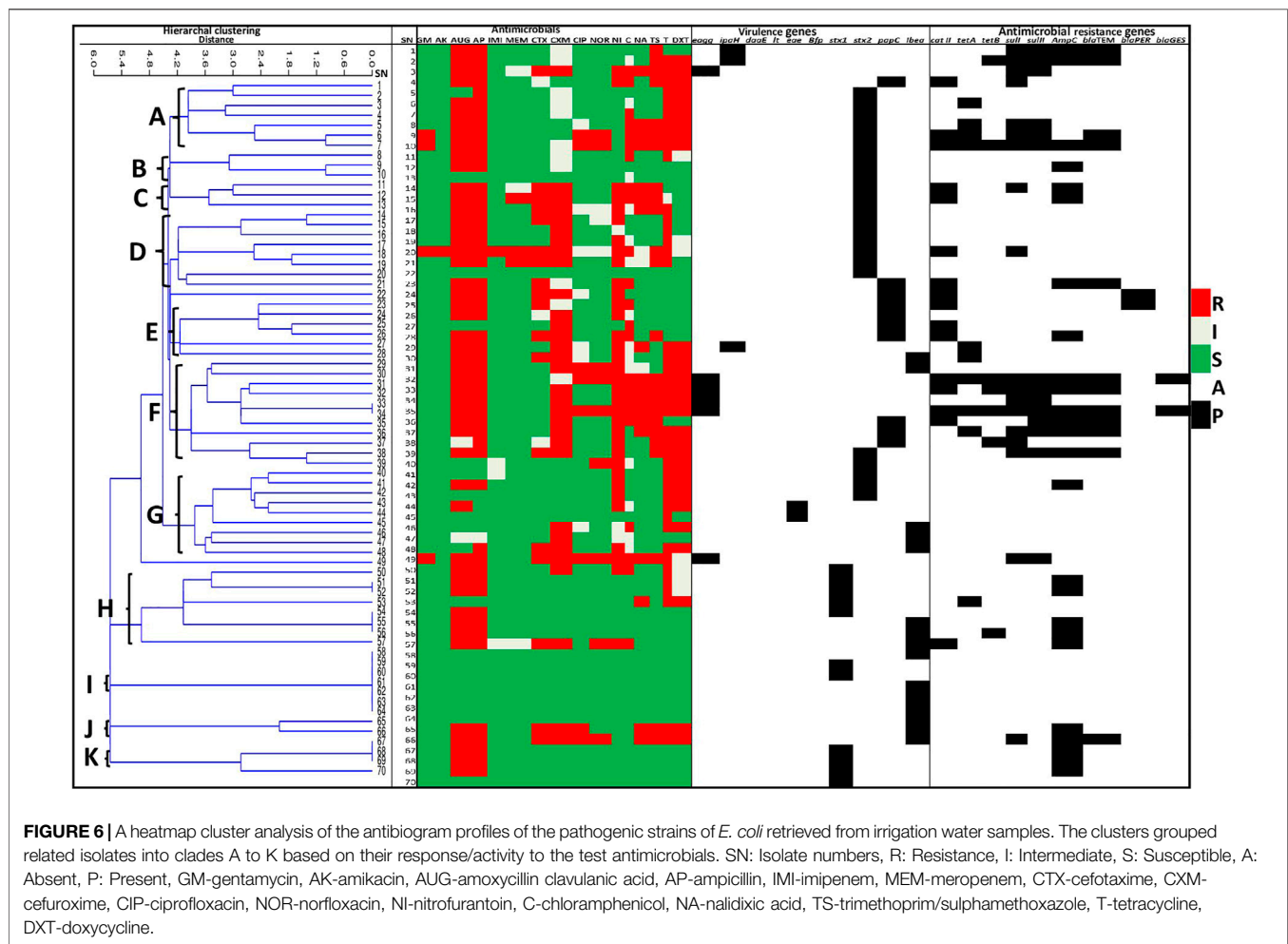
**FIGURE 5 |** The frequency of occurrence of ARGs detected in the pathogenic strains of *E. coli* recovered from irrigation water and agricultural soil. n: the number of isolates phenotypically resistant against the associated antimicrobial.

**TABLE 4 |** The MARGs of the pathogenic strains of *E. coli* recovered from irrigation water.

SN	MAR Genotypes	No. of ARGs	No. of non β-lactams and ESBLs	No. of β-lactams and ESBLs	No. of observed patterns
1	catII-AmpC	2	1	1	3
2	catII-blaPER	2	1	1	2
3	catII-sull	2	2	0	2
4	sull-sull	2	2	0	3
5	tetB-AmpC	2	1	0	1
6	tetB-sull	2	2	0	1
7	catII-AmpC-bla <sub>TEM</sub>	3	1	2	1
8	catII-sull-AmpC	3	2	1	1
9	tetA-sull-sull	3	3	0	1
10	sull-AmpC-bla <sub>TEM</sub>	3	1	2	1
11	catII-sull-AmpC-bla <sub>TEM</sub>	4	2	2	1
12	sull-sull-AmpC-bla <sub>TEM</sub>	4	2	2	2
13	catII-tetA-sull-sull-bla <sub>TEM</sub>	5	4	1	1
14	tetA-sull-sull-AmpC-bla <sub>TEM</sub>	5	3	2	1
15	tetB-sull-sull-AmpC-bla <sub>TEM</sub>	5	3	2	1
16	catII-tetB-sull-sull-AmpC-bla <sub>TEM</sub>	6	4	2	1
17	catII-tetA-tetB-sull-sull-AmpC-bla <sub>TEM</sub>	7	5	2	1
18	catII-tetA-tetB-sull-sull-AmpC-bla <sub>TEM</sub> -bla <sub>GES</sub>	8	5	3	2

**TABLE 5** | The MARGs of the pathogenic strains of *E. coli* recovered from agricultural soil.

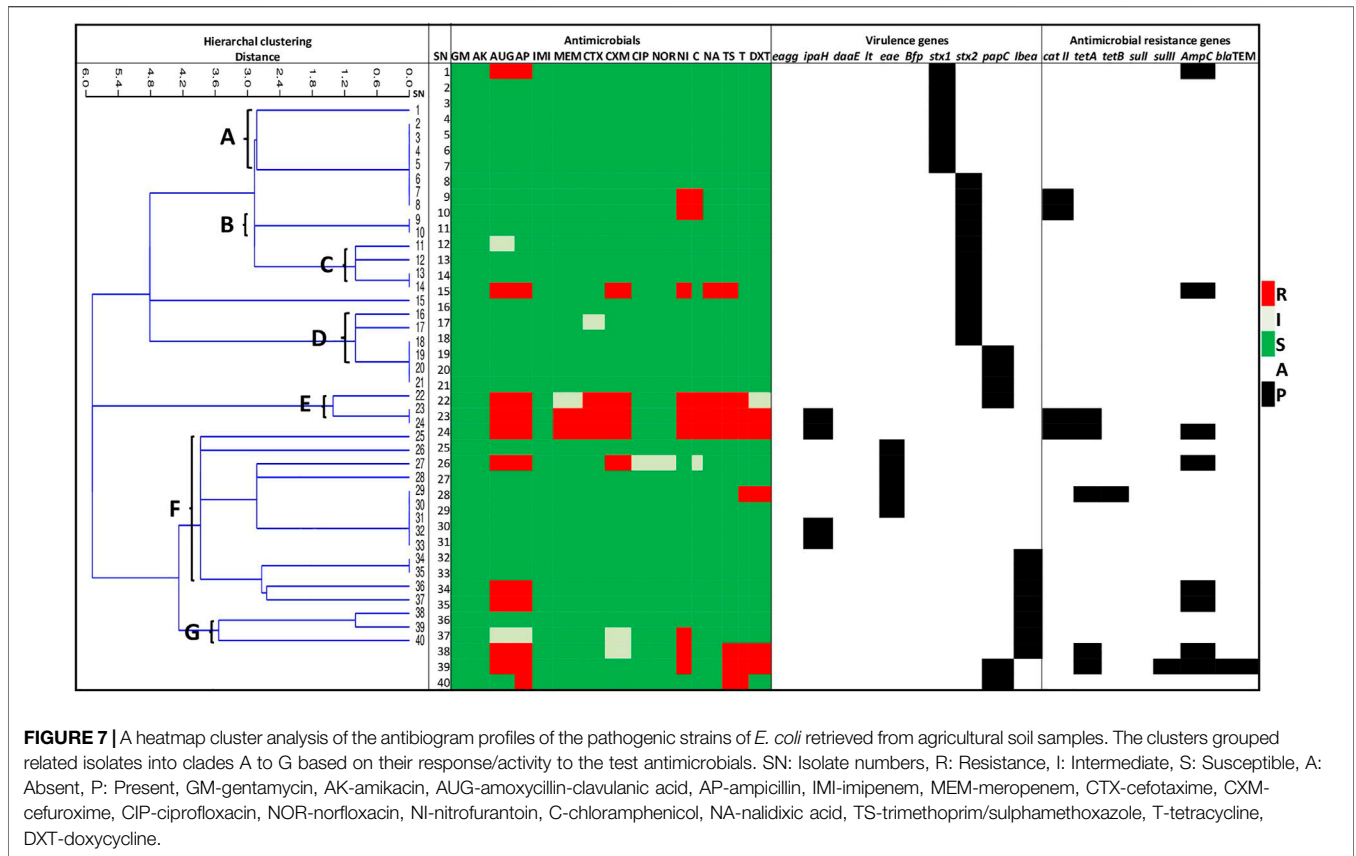
SN	MAR Genotypes	No. of ARGs	No. of non $\beta$ -lactams and ESBLs	No. of $\beta$ -lactams and ESBLs	No. of observed patterns
1	<i>catII-tetA</i>	2	2	0	1
2	<i>tetA-tetB</i>	2	2	0	1
3	<i>tetA-AmpC</i>	2	1	1	1
4	<i>catII-tetA-AmpC</i>	3	2	1	1
5	<i>tetA-sullI-AmpC-bla<sub>TEM</sub></i>	4	2	2	1



EPEC, EHEC and 2 ExPEC including UPEC and NMEC were detected in the agricultural soil samples in this study. This poses a huge risk to food safety and public health considering the role the preharvest environmental niches play in the transmission of infectious disease pathogens to the food web (Iwu and Okoh, 2019).

The prevalence of *E. coli* pathotypes and their associated virulence genes in irrigation water samples ranged from 2.9% (EPEC/*eae*) to 32.9% (EHEC/*stx2*) while that in agricultural soil samples ranged from 10% (EIEC/*ipaH*) to 27.5% (EHEC/*stx2*). This implies that EHEC encoded by the *stx2* genes were

predominantly detected in this study. This contrasts a similar study in Johannesburg, South Africa whereby EHEC at a prevalence rate of 3% was the least prevailing pathotype in environmental water samples while EPEC was the most prevailing pathotype at a prevalence rate of 19.2% (Bolukaoto et al., 2021). Another contrasting finding in South Africa indicated that EAEC encoded by the *aatA* and/or *aaiC* genes was the predominant pathotype in irrigation water and food products at a prevalence rate of 2% (Aijuka et al., 2018). These contrasts may be attributed to the differences in the geographical spread of *E. coli* pathotypes which is largely impacted by the



varying levels of anthropogenic activities specific to the study areas. Considering that EHEC is generally involved in large outbreaks globally (Torres, 2017), detecting them at a high prevalence rate in the preharvest environment in this study is a ticking time bomb for fresh produce related disease outbreaks. It also indicates that the study sites are potential hotspots for the spread of pathogenic strains of *E. coli* with outbreak potential. This requires urgent attention as EHEC does not only cause diarrheal disease but also cause medical complications such as hemorrhagic colitis and hemolytic uremic syndrome among the human population (Torres, 2017).

The emergence of antimicrobial resistant pathogenic bacteria has prompted the need to continually investigate their presence in the environment, from where they can reach humans. The pathogenic strains of *E. coli* retrieved from the preharvest environmental samples in this study conferred a high resistance against the  $\beta$ -lactams including ampicillin and amoxicillin-clavulanic acid, tetracyclines, nitrofurantoin and trimethoprim/sulphamethoxazole. This implies that these antimicrobials are indiscriminately used in this study area. A similar study in Texas, United States recorded a high resistance against ampicillin among 81% of their isolates (Summerlin et al., 2021). Additionally, a similar study in Spain recorded a high resistance against sulfamethoxazole, ampicillin and tetracycline (Amato et al., 2021). On the flip side, the pathogenic strains of *E. coli* retrieved from the preharvest environmental samples in this study were highly susceptible to aminoglycosides including amikacin and gentamicin,

carbapenems including imipenem and meropenem, fluoroquinolones including ciprofloxacin and norfloracin, cepheims including cefotaxime and quinolones including nalidixic acid. Fortunately, the median scores of the SARI of the *E. coli* pathogens recovered from the preharvest environmental samples in this study were low. This indicates that the isolates conferred resistance against the test antimicrobials at a low rate. In other words, only a few of the isolates were resistant against the test antimicrobials. The median SARI score of the *E. coli* pathogens recovered from irrigation water was significantly higher than that of the *E. coli* pathogens recovered from agricultural soil. This indicates that the irrigation water are better reservoirs of antimicrobial resistant bacteria. This is attributed to the numerous anthropogenic activities observed at the irrigation water sources which included animal intrusion, recreational activities, dumping of refuse, discharge of poultry and swine effluents and discharge of WWTP effluents. Additionally, the irrigation water sources across all the sampling sites aside S12 and S17 were surface water bodies which are open to various contamination sources.

Although the *E. coli* pathovars recovered from the preharvest environment in this present study had relatively low SARI, most of the resistant isolates were highly multidrug resistant against the test antimicrobials. The MDR *E. coli* pathovars in irrigation water samples were majorly detected in S5 and S18. This is not surprising as the irrigation water sources in these sampling sites receives effluents from livestock farms and WWTPs. Interestingly, no MDR *E. coli* pathovar were detected in irrigation water samples



collected from S12 and S17. This is probably because the source of irrigation water in these sampling sites were treated municipal water and borehole, indicating that they are the ideal sources of water required for the primary production of fresh produce. In the same vein, The MDR *E. coli* pathovars in agricultural soil samples were detected in S5 (loamy soil), S7 (clay soil) and S18 (loamy soil). The agricultural soil in these sampling sites were found to be amended using poultry and swine faeces and organic composts of animal origin. This may have attributed to the occurrence of the MDR *E. coli* pathovars in the agricultural soil in the sampling sites.

The majority of the MARP patterns of the MDR isolates in the preharvest environmental samples occurred uniquely while only a few occurred in duplicates. This suggests that each MDR isolate in the preharvest environment were exposed to unique, yet highly diversified antimicrobial selective pressure which may have led to the rapid evolution and transmission of diverse resistance determinants between the isolates. The MARI of all the MDR isolates in this study were all greater than the benchmark of 0.2. This confirms that antimicrobials are indiscriminately used in the study area. As a result, the preharvest environments in the study area are high-risk environments that favour the evolution and dissemination of MDR pathogens. This is plausible as antimicrobials dispensed to humans and animals are often not completely broken down. The metabolites are discharged into the environment which eventually finds their way to the preharvest environmental niches such as irrigation water and agricultural soil where they cause natural selective pressure for the resident bacteria (Iwu et al., 2020). There was no significant difference between the MARI of the *E. coli* pathogens in irrigation water and that of the *E. coli* pathogens in agricultural soil. This indicates that irrigation water and agricultural soil are equally high-risk environments as they are great receptacles of antimicrobial metabolites.

The presence of antimicrobials in the environment encourages the horizontal transfer of antimicrobial resistance genes between related and unrelated bacteria. This increases the prevalence of resistant bacteria in the environment and exacerbates the risk of super-resistant infections (Lerminiaux and Cameron, 2019). In this study, *sulI* sulfonamide resistance-encoding gene was the most prevailing ARG in the pathogenic strains of *E. coli* retrieved from irrigation water samples while *catII* phenicol resistance-encoding gene and *AmpC*  $\beta$ -lactam resistance-encoding gene were the most prevailing ARGs in the pathogenic strains of *E. coli* retrieved from agricultural soil samples. The *tetA* and *tetB* tetracycline resistance-encoding genes were also detected from the pathogenic strains of *E. coli* in both irrigation water and agricultural soil at a relatively high prevalence rate. These genes including macrolide and fluoroquinolone resistance encoding genes have been identified as the most abundant ARGs in the aquatic environments (Zhai et al., 2016; Christou et al., 2017; Dang et al., 2017). Despite the scarcity of studies on the prevalence of ARGs in agricultural soil, the abundance of the aforementioned ARGs in the preharvest environment is not surprising since the associated antimicrobials (sulfonamides, phenicols, tetracyclines, and ampicillin) are traditionally used as a first-line treatment of gastrointestinal infections. Additionally, the associated antimicrobials are used

as growth promoters in the Eastern Cape Province in South Africa (Iweriebor et al., 2015).

$\beta$ -lactams are the most widely used antimicrobials in the treatment of a wide variety of infectious diseases. This is because of their low toxicity and high effectiveness. This has led to an increased prevalence of the various variants of *AmpC* and ESBLs in the environment (Livermore, 1996; Iwu et al., 2021). The ESBLs in the pathogenic strains of *E. coli* detected in the preharvest environmental samples in this study were *bla*<sub>TEM</sub>, *bla*<sub>PER</sub>, and *bla*<sub>GES</sub>. This corroborates similar studies whereby *bla*<sub>TEM</sub> producing *E. coli* was detected in irrigation water and vegetables in household farms (Araújo et al., 2017) as well as in agricultural irrigation waters from Valencia city, Spain (Amato et al., 2021). Although *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>CTX-M</sub> have been identified as the most prevalent ESBLs in clinical and agricultural settings globally (Njage and Buys, 2015; Ye et al., 2017), our study, as a contrast, indicates the presence of *bla*<sub>PER</sub> and *bla*<sub>GES</sub> producing *E. coli* in the preharvest environment, hence the need for constant surveillance of the ESBLs in the environment. Sadly, a high proportion of the pathogenic *E. coli* in this study harboured multiple ARGs, some of which were a combination of non- $\beta$ -lactams/ESBLs with  $\beta$ -lactams/ESBLs. The majority of the MARGs patterns occurred uniquely, suggesting a rapid genetic exchange of ARGs between the pathogenic strains of *E. coli* in the preharvest environment.

Our study indicated a strong positive correlation between the genetic and phenotypic characteristics of the pathogenic strains of *E. coli* in the preharvest environment. This suggests that the detected ARGs were responsible for the resistance phenotypically observed among the isolates. Our antibiogram-based cluster analysis indicated that the isolates were highly distinct. This suggests that the preharvest environment is saturated with expanded classes of antimicrobials, which in turn causes a highly diversified AMR selective pressure for the evolution and dissemination of diverse multidrug resistant pathogenic *E. coli* strains and their determinants. The strength of this study is that it has provided more insight into the occurrence and diversities of multidrug resistant pathogenic strains of *E. coli* in the preharvest environment, an area that is still understudied despite its public health importance. The study also employed a robust statistical analysis to explore the epidemiological and correlational distribution of the multidrug resistant pathogenic strains of *E. coli* in the preharvest environment. The study, nonetheless, had a limitation. The grab sampling technique used in this study provided only a snapshot of the distribution of multidrug resistant *E. coli* pathovars in the preharvest environmental samples collected from the study sites.

## CONCLUSION

This study revealed the high levels of diversified multidrug resistant *E. coli* pathovars in the preharvest environmental samples including irrigation water and agricultural soil collected from fresh produce farms in two District Municipalities in the Eastern Cape Province, South Africa. These agricultural niches were found to be high risk environments that support the evolution and dissemination of AMR. EHEC encoded by the *stx2* toxin gene was the most prevalent pathovar in the preharvest environment indicating an outbreak

potential when transferred to the food web. This calls for a multisectoral and evidence-based approach to minimize the occurrence of multidrug resistant pathogenic bacteria in the preharvest environment and their potential spread to the food web.

## 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

Conceptualization: CI and AI; methodology: CI and AI; investigation: CI; data curation: CI; data analysis: CI; writing—original draft preparation: CI; writing—review and editing, CI, AK, BI, and AI; supervision: AI; funding

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The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2022.858964/full#supplementary-material>

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