

Occurrence and antibiogram signatures of members of the Enterobacteriaceae family recovered from vegetables, river water and hospital effluents in Amathole and Chris Hani District Municipalities in the Eastern Cape Province, South Africa.

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Abstract

Background

Rising incidences of antimicrobial resistance has become a major public health issue globally resulting in increasing health-care costs and severe and lethal diseases. Several reports have documented alarming increases of antimicrobial resistance in the Enterobacteriaceae family, hence, this study evaluated the occurrence and antibiogram signatures of Enterobacteriaceae isolates recovered from vegetables, hospital effluents and river water samples in two district municipalities in the Eastern Cape Province, South Africa.

Results

Out of 142 presumptive isolates, 105 were confirmed to belong to the Enterobacteriaceae family. From these, 45% were identified as *E. coli*, 24% as *Enterobacter* spp., 20% as *Citrobacter* spp. and 11% as *Klebsiella* spp. All the isolates demonstrated high resistance against ampicillin at a frequency of 91.4%, followed by nalidixic acid (86.7%), tetracycline (82.9%), cefuroxime (81.9%) and doxycycline (81.9%). The beta-lactam resistance gene *bla*_{TEM} was detected in 77.8% of the *E. coli* isolates and 33.3% of the *Klebsiella* isolates. The *sul1* gene was detected in 23.1% of the *Enterobacter* species, while *sul2* gene was detected in 70% of the *Klebsiella* species. All the *Enterobacter* species were positive for the *strB* gene.

Conclusions

We conclude that the vegetables, hospital effluents and river water in the two District Municipalities are reservoirs of multidrug resistant members of the Enterobacteriaceae family and a potential health hazard for consumers.

Background

The emergence, spread and increase of resistance against conventional antibiotics has taken a centre stage in therapeutic medicine globally, especially in the low-income Asian and African countries, where health infrastructure and facilities are still largely underdeveloped [1], and this has become a universal health problem, accountable for the increasing incidences of severe and fatal illnesses [1]. Among the three communal human health threats reported in 2011, the World Health Organization (WHO) adjudged antimicrobial resistance (AMR) as a huge threat to human health [2]. Unabated increase of AMR hinders clinicians' choices of therapeutic options, making medical procedures such as hip arthroplasty, chemotherapy, organ transplants, hemodialysis and care for preterm babies more complicated [3]. Illnesses linked to antimicrobial-resistant infectious agents contribute significantly to the escalating costs of health care as they occasion lengthen hospital stay and medical bills [4].

The increasing resistance in bacterial species has been linked primarily to mobile genetic elements, which can spread rapidly through bacterial populations [5]. The leading cause of antimicrobial resistance and its transmission is reported to be the inappropriate and extensive usage of antimicrobial agents, including therapeutic and non-therapeutic usage of antimicrobial agents in the production of livestock [6]. Other factors contributing to the rise of AMR include inadequate hygiene, lack of sanitation precautions, and lack of prevention and control of infection in hospital environments. All these factors impact on human health care and environment [7, 8, 9, 10].

One of the major mechanisms of resistance is the production of extended-spectrum beta-lactamases (ESBLs) [11, 12]. These enzymes promote resistance of bacteria against several classes of antimicrobials including second and third-generation cephalosporins, penicillins, monobactams and some fluoroquinolones [13, 14]. According to [15], microorganisms having proficiency of producing ESBL may initiate infections, which results in undesirable consequences such as reduced rates of clinical and microbiological responses to treatments, extended hospitalization, as well as huge hospital expenditures.

Increasing antimicrobial resistance has been reported in Enterobacteriaceae; hence, this family has become an important challenge in disease control [16, 17, 18, 19]. Despite the enormous public health challenge posed by this bacterial family, limited information is available on their occurrence and characteristics in vegetable and water resources in the Eastern Cape Province, South Africa. Hence, this study investigated the occurrence and antibiogram signatures of some members of the Enterobacteriaceae family recovered from vegetables, hospital effluents and river water in Amathole and Chris Hani District Municipalities (DMs) in the Eastern Cape Province, South Africa.

Results

Prevalence of four members of the Enterobacteriaceae family

The prevalence of four members of the Enterobacteriaceae is represented in Fig. 1. A total of 105 presumptive isolates were identified as *E. coli* (45%), *Enterobacter* spp. (24%), *Citrobacter* spp. (20%) and *Klebsiella* spp. (11%).

Antimicrobial resistance and phenotypic characteristics

The antimicrobial susceptibility patterns of four members of the Enterobacteriaceae family are presented in Fig. 2. Generally, very high resistance frequencies were observed against ampicillin (91.4%), followed by nalidixic acid (86.7%), tetracycline (82.9%), cefuroxime (81.9%) and doxycycline (81.9%) (Fig. 2). The least resistance was observed against imipenem (27.6%), followed by gentamycin (30.5%) and amikacin (35.2%). All the *Enterobacter* spp. showed resistance against amoxicillin/ clavulanate acid, while all the *Citrobacter* spp. also showed resistance against ampicillin, cefotaxime, nalidixic acid and doxycycline. About 89% of the *E. coli* isolates exhibited resistance against tetracycline.

Multiple antibiotic resistance phenotypes (MARPs) and multiple resistance index (MARI) of the confirmed isolates

There were four predominant MARPs observed among the isolates which includes AUG-AP-CTX-CXM-CIP-NI-C-CO-NA-TS-T-DXT (13%), GM-AUG-AP-CTX-CXM-CIP-NOR-NI-C-PB-CO-NA-TS-T-DXT (8%), AUG-AP-CTX-CXM-CIP-NOR-NI-C-PB-CO-NA-TS-T-DXT (13%), AUG-AP-CTX-CXM-CIP-NOR-NI-PB-CO-NA-TS-T-DXT (13%) and AK-AUG-AP-CTX-CXM-NI-C-PB-CO-NA-TS-T-DXT (13%). The highest MARI value obtained was 0.9 exhibited by 3% of the isolates and these isolates were resistant against ≤ 17 antibiotics (Table 1).

Table 1: Multiple antibiotic resistance indices of the confirmed isolates

MARI value	Frequency of isolates (%)	Number of antibiotics
0.16	3	3
0.22	2	4
0.28	3	5
0.33	4	6
0.39	2	7
0.44	6	8
0.5	5	9
0.56	8	10
0.61	11	11
0.67	13	12
0.72	13	13
0.78	13	14
0.83	8	15
0.89	8	16
0.94	3	13

Detection of antibiotic resistance genes

All the confirmed isolates harboured all of the beta-lactam resistant genes assayed. In addition, the sulphonamides, aminoglycosides and tetracyclines resistant genes were detected among the isolates. *E. coli* isolates exhibited high prevalence of *bla*_{TEM} (77.8%) compared to the other three target bacteria. In contrast, *Klebsiella* spp. exhibited the least

frequency of *bla*_{TEM} (33.3%) (Table 2). Only *Klebsiella* species were positive for *bla*_{SHV} at a frequency of 16.7%, while *sul1* was detected in 23.1% of the *Enterobacter* species. The *Klebsiella* species were positive for *sul2* at a frequency of 70%. Tetracycline resistance gene, *tetA* was also detected in 33.3% of the *Klebsiella* spp. while *tetM* was detected in 28.6% of the *E. coli* isolates.

Table 2: Frequency of detection of resistance genes in the isolates.

Resistance Gene	<i>E. coli</i>	<i>Enterobacter</i> spp.	<i>Citrobacter</i> spp.	<i>Klebsiella</i> spp.
<i>bla</i> _{TEM}	77.8%	44%	76.2%	33.3%
<i>bla</i> _{SHV}	0	0	0	16.7
<i>bla</i> _{OXA-1}	13.3%	8%	4%	0
<i>bla</i> _{CTX-M-2}	11.1%	36%	23.8%	16.7%
<i>bla</i> _{CTX-M-9}	8.8%	8%	0	8.3%
<i>bla</i> _{DHA}	13.3%	0	0	0
<i>bla</i> _{GES}	11.1%	8%	23.9	16.7%
<i>bla</i> _{OXA-48}	2.2%	0	12%	8.3%
<i>sul1</i>	8.8%	23.1%	15%	0
<i>sul2</i>	29.9%	30.8%	10%	70%
<i>TetA</i>	17.8%	19%	10%	33.3%
<i>TetM</i>	28.6%	19%	19%	0
<i>AadA</i>	100%	86%	81%	100%
<i>StrA</i>	70%	100%	28.6%	25%
<i>StrB</i>	27%	57.1%	33.3	100%
<i>aacA2</i>	30%	71.4%	9.5%	100%
<i>aphA1</i>	13.3%	28.6%	4.8%	25%
<i>aphA2</i>	13.3%	71.4%	14.3%	0

Discussion

The prevalence of four important members of the Enterobacteriaceae family in this study followed the order *E. coli* (45%), *Enterobacter* spp. (24%), *Citrobacter* spp. (20%) and *Klebsiella* spp. (11%). The occurrence of these organisms in the different samples suggests exposure to faecal contamination, and may also indicate the possible presence of other pathogens such as *Vibrio* spp. and *Campylobacter* spp. [20]. The occurrence of the Enterobacteriaceae in the samples raise a particular concern as these microorganisms have a potential for causing severe infections such as diarrhoea, pneumonia, urinary tract infection, meningitis and many more [21]. Also, the occurrence of the organisms in vegetable samples presents a problem to public health because some of these fresh produce are usually consumed

with little or no cooking. The presence of Enterobacteriaceae in rivers and hospital effluents is worrisome. The bacteria could persist and subsequently be transferred along the human food chain [22]. Furthermore, the presence of these microbes in river water might be due to the discharge of untreated hospital sewage systems [23], or discharge of agricultural, animal, domestic, human and industrial wastes [24, 25].

Several factors are driving the contamination of fresh produce by Enterobacteriaceae. Sources of contamination in fresh produce might include application of animal manure, direct contact with infected worker during harvesting, unhygienic handling and processing. Also, untreated irrigation water may contribute to contamination of vegetables [26], and transportation vehicle have also been implicated as a source of contamination of vegetables by microorganisms. Also, cultivation of fresh produce on contaminated soil could lead to contamination of the produce [27].

A relatively large number of the isolates recovered in this study exhibited resistance against one or more test antibiotics, suggesting high frequency of antimicrobial resistance in the host communities. All the bacterial isolates showed high frequency of resistance against ampicillin, tetracyclines, beta-lactams and sulphonamides (Fig. 2). Tetracycline resistance has been frequently reported from environmental samples [28, 29, 30], and could be due to the abuse of tetracycline in animal feeds as a growth promoter [31].

Also, there is high frequency of resistance against ampicillin, tetracycline, cefuroxime and cefotaxime in all the four bacterial types and corroborates reports from previous studies [32, 33, 34]. High susceptibility of *Klebsiella* spp. (83%) against imipenem was observed in this present study, which is contrary to the report of [35] who reported high resistance of *Klebsiella* spp. against imipenem, colistin and polymyxin B. These antimicrobials are recommended to be antimicrobials of last resort for treating bacterial infections. Also, [36] reported that all their *E. coli* isolates were resistant against colistin. According to [37], for many decades, colistin was not recommended for use due to its toxicity and the availability of other safer antimicrobials such as penicillins. However, the use of colistin has now been included in therapeutic options due to the rise in antimicrobial resistance.

The health risk associated with the transmission of antimicrobial resistance in the environment was evaluated using multiple antibiotic resistance index (MARI). MARI values ranged between 0.16 and 0.94 (Table 1). The MARI value greater than 0.2 suggests that isolates are recovered from an environment with high usage of antibiotics [38]. About 97% of the test bacterial isolates had a MARI estimate greater than 0.2 and this suggests that these isolates were exposed to high antibiotic pressure, which might have stemmed from misuse of antibiotics in the selected study areas [39, 1]. The test bacterial isolates were resistant to more than one test antibiotics and this indicates that these isolates are multi-drug resistant. Multidrug-resistance raises a health concern because it limits the treatment options available for bacterial infections [1]. Multidrug-resistance leads to re-emerging of certain diseases, which are associated with health implications such as prolonged illness period, higher cost for therapy, and increased risk of death [1].

Antibiogram signature of the bacterial isolates revealed the occurrence of eighteen antibiotic resistance genes conferring resistance against different antibiotic classes. The most prevalent antibiotic resistant gene detected among the isolates was *bla*_{TEM} among the other genes conferring beta-lactam resistance. The findings from this study are inline previous studies regarding the frequency of *bla*_{TEM} in members of Enterobacteriaceae family [40, 41, 42]. The isolates also exhibited frequent occurrence of other antibiotic resistant genes including *aacA2*, *aadA*, *strA* and *strB* which confer against aminoglycosides. This raises a particular concern, as aminoglycosides are one of the highly potent, broad-spectrum antimicrobials that have been frequently prescribed for treating life-threatening infections for several decades. The commonest mechanism of aminoglycosides resistance document include the production of *N*-acetyltransferases (AAC), nucleotidyltransferases (ANT) and *O*-phosphotransferases (APH), which are enzymes that modify the antibiotic, thus rendering the antibiotic inactive [43, 44, 45]. For instance the AAC enzyme acetylate the amino group at the 6'-position of the aminoglycoside antibiotic while APH, phosphorylate the hydroxyl group at the 3'-position of aminoglycosides and disrupt the binding of the aminoglycoside antibiotic to the 16S rRNA molecule of the target organism [45].

The *tetA* and *tetM* resistant genes conferring tetracycline resistance were detected in the recovered isolates. Tetracycline resistance genes like *tetM* disallow the binding of tetracycline antibiotic to the ribosome by producing elongation factor-like ribosomal protection proteins that stabilize ribosome transfer RNA interactions in the presence of tetracycline molecules [46]. Occurrence of tetracycline resistance genes in Enterobacteriaceae have been previously reported [47, 48, 49, 50]. Interestingly, only *Klebsiella* species that demonstrated high occurrence of *sul2* gene at 70% proportion, while *sul1* was not detected in this organism. Surprisingly, *E. coli*, *Citrobacter* species and *Enterobacter* spp. displayed low occurrence of both *sul1* and *sul2* genes even though they displayed high resistance against trimethoprim antibiotic. This might suggest that resistance against trimethoprim was mediated by other resistance mechanisms such as efflux pump, the ability to form biofilm and possession of integrons, which were not assayed in the present study.

Conclusions

Results from this study clearly showed that vegetables, river water and hospital effluents in the study communities are major potential reservoirs of antimicrobial-resistant Enterobacteriaceae and antimicrobial-resistance genes. The occurrence of Enterobacteriaceae in the samples suggests the potential presence of other pathogens and also indicate faecal contamination. Our findings highlight the importance of antibiotic resistance surveillance in fresh produce, hospital effluents and freshwater resources in the host communities to avoid the spread of more resistant pathogens and indorse strategies for the therapeutic management of multi-drug resistant infections.

Materials And Methods

Study designs and source of samples

Twelve vegetables, hospital effluents (2) and river water (6) samples were aseptically collected between October and November 2017. The samples were collected from Amathole and Chris Hani DMs in the Eastern Cape Province, South Africa. Sampling sites in Chris Hani DMs are located within geographical coordinates 32.0348° S, 27.8165° E and 32°48'37" S; 26°52'20" E, while sample sites in Amathole District Municipality were located within 32°47'17" S; 26°50'31" E and 32°53'23" S; 27°23'17" E.

Isolation and identification

Samples were analysed by culture-based methods following the descriptions of [51,52]. Presumptive *E. coli*, *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. were recovered on Eosin methylene blue agar (Laboratories CONDA, South Africa) incubated at 37 °C for 24 hours. Presumptive *E. coli* isolates appeared as green metallic sheen colonies while large mucoid blue to purple colonies were selected as presumptive *Klebsiella* spp. Growth of presumptive *Citrobacter* spp. and *Enterobacter* spp. were recognized by large mucoid red colonies. The identities of the presumptive isolates were confirmed using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF MS).

Antimicrobial resistance profiling of confirmed Enterobacteriaceae

The confirmed isolates were screened for their antimicrobial susceptibility/resistance patterns using Kirby Bauer disk diffusion method as described by CLSI (2018) against a panel of eighteen commercial antibiotic disks (Davies Diagnostics (Pty) Limited, S.A.) cutting across eleven families of antimicrobials which comprised of the amino-glycosides [amikacin (30 µg), gentamycin (10µg)]; cepheims: cefuroxime (30 µg)]; carbapenems [meropenem (10µg), imipenem (10µg)]; fluoroquinolones [ciprofloxacin (5µg), norfloxacin (30µg)]; quinolones [nalidixic acid (30µg)]; sulphonamides [trimethoprim (25µg)]; nitrofurantoin [nitrofurantoin (300µg)]; phenicols [chloramphenicol (30µg)]; tetracyclines [tetracycline (30µg) and doxycycline(30µg)]; cephalosporins [cefotaxime (30 µg)]; beta-lactamases [combination discs of amoxicillin/clavulanate (30/10 µg) and ampicillin (25µg)]; polymyxins [polymyxin B (300µg) and colistin (25µg)]. Diameters of the zone of inhibition were measured and compared to the Clinical and Laboratory Standards Institute (CLSI) interpretative charts of 2018 to determine the profiles of the isolates. Multiple antibiotic resistance profile was defined as the resistance against three and more different classes of antibiotics, while multiple antibiotic resistance indices (MARI) of the isolates were estimated as previously described by [53]. $MARI = a/b$; in which "a" denotes the total number of resistance obtained and "b" denotes total number the number of the used antibiotics.

Detection of antimicrobial resistance genes

Detection of relevant antimicrobial resistance genes was done using polymerase chain reactions technique with specific primers for antimicrobial-resistant genes. Eighteen antibiotic resistance genes cutting across different classes of antibiotics were assayed and these include *strA*, *strB*, *aadA* [54], *aacA2*, *aphA1*, *aphA2* [55] for the aminoglycosides], *tetA*, [56], *tetM* [57] for the tetracyclines, *sul1* [55] and *sul2* [58] for the sulphonamides and beta-lactam resistance genes *bla_{TEM}*, *bla_{SHV}*, *bla_{OXA-1}*, *bla_{CTX-M-2}*, *bla_{CTX-M-9}*, *bla_{DHA}*, *bla_{GES}* and *bla_{OXA-48}* [59].

Abbreviations

WHO

World health organisation

AMR

Antimicrobial resistance

ESBL

Extended spectrum beta-lactamase

DM

District Municipality

MARP

Multiple antibiotic resistance phenotypes

MARI

Multiple antibiotic resistance index

CLSI

Clinical laboratory standards institute guidelines

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable as the manuscript does not contain any data from any other person.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LM was the main researcher and contributed to the collection of data, interpretation, and analysis of results and prepared the manuscript and is the corresponding author. AM, NN and AIO oversaw the study, contributed to the analysis and interpretation of data, and drafted the manuscript. All authors read and approved the final manuscript.

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Figures

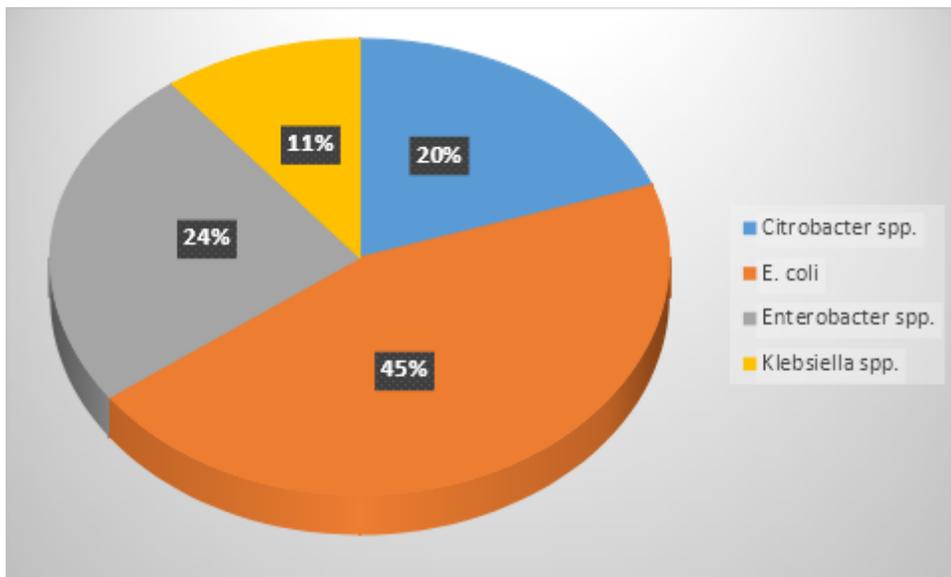


Figure 1

Prevalence of some members of the Enterobacteriaceae family.

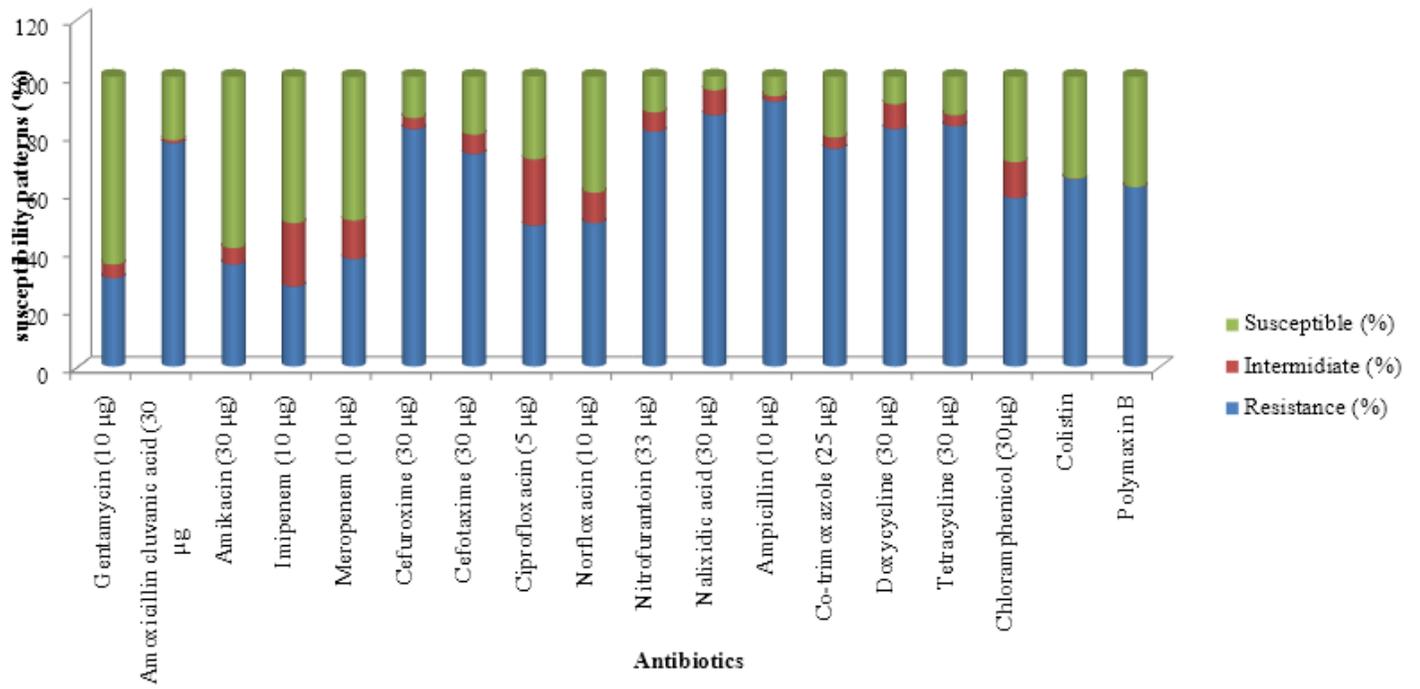


Figure 2

Antibiogram profiles of confirmed isolates from vegetables, hospital effluents and river water samples sourced in Amathole DM and Chris Hani DM