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The Emergence of Colistin- and Imipenem-Associated Multidrug Resistance in *Escherichia coli* Isolates from Retail Meat

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Abstract

To determine the prevalence of *Escherichia coli* and their drug resistance profiles in fresh pork sold at two retail outlets (open-air market and closed retail stores) in Alice, South Africa. Retail meat samples (n = 176) collected from four shops (two from open-air markets and two from closed stores) were analyzed by conventional biochemical and PCR-based molecular confirmatory tests. The confirmed isolates were profiled for antimicrobial susceptibility to a panel of 12 commercial antibiotics: tetracycline, ampicillin, sulphamethoxazole trimethoprim, erythromycin, gentamycin, colistin sulphate, cefotaxime, chloramphenicol, norfloxacin, ciprofloxacin, cefuroxime, and imipenem. Colistin, ampicillin, and erythromycin resistance genes were profiled with the gene-specific primers. Multidrug resistance (MDR) and the association of imipenem and colistin in the MDR profile were determined. A total of 68 (39.08%) *E. coli* isolates were confirmed by PCR analysis. Resistance was most common to erythromycin (100%), followed by cefotaxime (95.58%), ampicillin (88.23%), cefuroxime (88.23%), trimethoprim-sulphamethoxazole (88.23%), and tetracycline (60.29%). Overall, 27/68 (39.70%) were MDR (≥3antibiotics classes). MDR *E. coli* isolates associated with imipenem resistance (50.00%) and colistin resistance (33.82%) were detected. The resistance genes were detected among the isolates though not in all the phenotypically resistant isolates. The detection of colistin resistance among MDR *E. coli* isolates from retail meat is troubling as the drug is a last resort antibiotic. Overall, the epidemiological implications of the findings are of public health importance.

Keywords: *Escherichia coli*, pork, multiple antibiotic resistance, susceptibility, retail outlet, colistin resistance, imipenem resistance

Introduction

Foodborne pathogens from animal sources account for most of the foodborne illnesses each year. Poor hygienic practices are a major contributing factor in most developing countries of Africa, with contaminated raw meat as a leading source of foodborne illness. Raw meats are available in the open air, retail shops, and abattoirs. Microbial contamination of food can therefore transpire at numerous phases across the food chain, which includes production, slaughtering, distribution, and retail marketing (Elhadi 2014). Foodborne diseases are burdensome and represent a significant world health problem. Globally, the microbiological safety of food is a growing public health challenge. By estimate, every year, 600 million, or nearly 1 in 10 people globally, become ill through the consumption of contaminated food. A total of 420,000 individuals out of this figure die, including 12,000 children who fall under 5 as reported by the World Health Organization's estimates on the global burden of foodborne diseases (WHO 2015). *Escherichia coli*, a member of intestinal microbiota, is potentially pathogenic organism for humans and animals (Bumunang et al. 2019). The presence of *E. coli* in meat indicates poor hygienic practices in abattoirs or retail outlets. Contaminated, uncooked or undercooked red meats are particularly important in transmitting these foodborne pathogens (Wang et al. 2012). Foods of animal origin have been implicated as a leading vehicle involved in foodborne diseases (Baek et al. 2009). Pork is a highly consumed red meat in the world (Joy et al. 2014; Grobbelaar et al. 2021), and Statista GmbH has projected that the global consumption will amount to around 127.2 million metric

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tons by 2029 (Grobbelaar et al. 2021). The pork consumption in South Africa was reported to increase from 3.5 kg (2000) to over 4.2 kg (Streicher 2012).

In South Africa, pork is partly responsible for around 16.3% of the gross value of agricultural production (Mohlatlole et al. 2013). To meet production goals, antimicrobial agents are vital for the prophylaxis and treatment of many diseases in pork production (Ström et al. 2018). The most frequently administered antimicrobial agents in pork production include colistin, third and fourth-generation cephalosporins, and carbapenems. Colistin, a member of the antimicrobial agents, referred to as polymyxins, is a mixture of polymyxin E1 and E2 that are pentacationic lipopeptides and are bactericidal in action. Colistin is active against a wide range of Gram-negative bacteria and has no activity against Gram-positive bacteria due to the absence of an outer membrane. It is used in both human and veterinary medicine. However, it is only an empiric drug in treating infections with multidrug-resistant, extensively drug-resistant, and pan drug-resistant bacteria in humans (Magiorakos et al. 2012). In veterinary medicine, it is commonly used to treat food-producing animals from chicken to pigs, claves, cattle, cows, meat, and milk-producing sheep, goats, and rabbits. It is also used in aquaculture for the prevention and treatment of infections attributed to members of *Enterobacteriaceae* and other Gram-negative bacteria and used for growth promotion.

Imipenem is a beta-lactam antibiotic of the carbapenems class with a broad spectrum of activity. The mechanism of action involves the inactivation of the penicillin-binding proteins (PBP), thereby resulting in cell wall lysis or inhibition of its formation. They bind to a specific PBP (PBP-1) that results in more rapid lysis compared to other beta-lactams, thus resulting in higher bactericidal activity and a more prolonged post-antibiotic effect. Carbapenems have a broad spectrum of activity and are among the most active of all antibiotics. Their spectrum includes Gram-negative bacilli, including Enterobacteriaceae and Pseudomonas aeruginosa. Antibiotic resistance is one of the biggest threats to global health, food security, and development today (WHO 2020). Antibiotic resistance may develop naturally, but antibiotic misuse by humans and animal husbandry is accelerating the process. Currently, a growing number of infections are becoming harder to treat as the antibiotics used to treat them have become less effective. The consequences of antibiotic resistance lead to more extended hospital stays, higher medical costs, and increased mortality. There are four fundamental mechanisms of antimicrobial resistance: (i) enzymatic degradation of antibacterial drugs, (ii) alteration in the bacterial proteins that are antimicrobial targets, (iii) changes in membrane permeability to antibiotics, and (iv) intrinsic resistance caused by an absence of drug's target in the organism. Antibiotic resistance can be either plasmid-mediated or maintained on the bacterial chromosome.

Generally, resistance to colistin could be due to acquired resistance in which a once naturally susceptible organism modifies its cell surface, such as altering its lipopolysaccharide structure. Other known resistance mechanisms include capsular polysaccharide shedding, thus resulting in the trapping or binding of polymyxin or colistin, as in *Klebsiella pneumoniae*. In organisms such as *Acinetobacter baumannii*, there are two known mechanisms of resistance to colistin, which include loss of lipopolysaccharide production and the modification of the system that allows bacteria to respond to environmental conditions, which ultimately results in lipid modification and membrane permeability. The second most adopted mechanism is transferable colistin resistance mediated by a plasmid-coded gene, *mcr*. Currently, there are many variants of the gene since the first identification of the *mcr-1* gene in *E. coli* isolates from food animals and their meat in China between 2011 and 2014 and in *E. coli* and *K. pneumoniae* isolates collected in 2014 from humans in China (Liu et al. 2016; Skov and Monnet 2016).

The location of colistin resistance genes on transferable plasmids has resulted in the widespread dissemination of colistin resistance in various strains recovered from different sources (Aminov 2011; Popowska 2012). It leads to the development of multiple co-resistance or cross-resistance in bacteria (Gwida and El-Gohary 2015; Hoelzer et al. 2017). It affects both pathogenic bacteria and healthy microbiota, with the latter serving as a potential reservoir of resistance genes for pathogens. Multidrug-resistant pathogens can be transferred through the food chain weakening the potency of antimicrobials administered during infections (Brunelle et al. 2013). Thus, the widespread antibiotic resistance among foodborne pathogens undermines the successful treatment of infectious diseases (Yao et al. 2016) as antibiotics are rendered ineffective due to resistance leading to frequent treatment failures (Poirel et al. 2016). Most colistin-resistant positive isolates carry different resistance genes, including carbapenemase (Yao et al. 2016; Poirel et al. 2016) and other resistance genes (Turlej-Rogacka et al. 2018).

The evolution of carbapenem resistance has become rampant in Gram-negative bacteria, especially in intensive care units (ICUs), where the selective pressure exerted by antibiotics on bacterial populations is strong. This development is mediated by mutations or insertion sequences (ISs) that inactivate the gene coding for porin OprD, the specific portal of entry for carbapenems into these organisms. The reduced outer membrane permeability that results from OprD loss

usually causes an increase in the MICs of all the carbapenem molecules, including imipenem, meropenem, and doripenem (Fournier et al. 2013). In addition to this very prevalent mechanism, carbapenem-resistant bacteria may acquire numerous foreign genes encoding different classes of β-lactamases that are capable of hydrolyzing carbapenems to varying degrees. Similarly, there is an ever-growing number of bacterial isolates producing metallo-β-lactamase (MBL) (class B) that have been reported from many countries in recent years, indicating that these enzymes could become the predominant cause of carbapenem resistance in no distant future (Fournier et al. 2013).

Although colistin-resistant isolates are emerging and have been found in Africa, data on multidrug-resistant isolates exhibiting carbapenem and colistin resistance is scarce. Additionally, there is a dearth of data as regards the prevalence of multiple resistant *E. coli* contaminating pork in Eastern Cape, South Africa. Here we present data on multidrug-resistant *E. coli* isolates.

Experimental

Materials and Methods

Sample collection. The study was conducted in Alice. Alice is situated in the Eastern Cape, the "livestock" province of South Africa, which has about 46% goats, 28% sheep, 20% pigs, and 21% cattle (Caine et al. 2014). Alice was selected based on the meat consumption rate by this area's inhabitants. One hundred eighty raw pork samples were randomly purchased from open-air markets (Shops A and B) and closed retail stores (Shops C and D) located in Alice. The samples were individually packed in sterile plastic bags, marked, and then transported in cooler boxes with ice parks (4°C) to the University of Fort Hare Microbiology Laboratory for immediate processing. The study was carried out between May 2018 and October 2018.

Isolation of presumptive organisms. The modified method described by Bersisa et al. (2019) was adopted to isolate bacteria. Briefly, the raw meat samples were swabbed with a sterile cotton swab, and each swab sample was used to inoculate chromogenic agar plate (Merck, South Africa), and then the plates were incubated at 37°C for 48 h. About two to three separate distinct colonies were picked and were selectively plated onto chromocult tryptone bile X-glucuronide agar (TBX agar; Merck, South Africa), incubated aerobically at 37°C for 24 h. Each deep blue colony was selectively picked as presumptive *E. coli* isolate into LB broth and incubated for 24 h at 37°C. These presumptive isolates were stored at –80°C in 25% glycerol until further analyses.

DNA extraction. Genomic DNA of the presumptive *E. coli* isolates was prepared by the method earlier reported by Iweriebor et al. (2015). Briefly, the presumptive isolates in glycerol stock were resuscitated overnight in LB broth, from which 2 ml was centrifuged at 10,000 rpm. The supernatant was discarded, the pellet resuspended in nuclease free water, boiled at 100°C, and the resultant supernatant was used as DNA template in all the PCR assays.

Molecular identification of presumptive *E. coli* by PCR. Isolates considered as presumptive *E. coli* were confirmed by PCR using the *uid*A oligonucleotide primer (Inqaba Biotech, South Africa) and the details of the sequences are as follows: F: 5'-AAAACGGCAAGAAAAAGCAG-3' and R: 5'-ACGGTGGTTAACAGTCTTGCG-3' (Tsai et al. 1993). Each PCR was performed as previously described by Iweriebor et al. (2015), followed by gel electrophoresis on 1.5% agarose and documented in a gel documentation system. *E. coli* ATCC® 25922™ reference strain served as a positive control in this study.

Determination of antimicrobial susceptibility of the isolates. Antimicrobial susceptibility patterns of the confirmed *E. coli* isolates were performed on Mueller-Hinton agar (MHA) plates (Oxoid CM337), as previously reported by Bauer et al. (1966). About four to five *E. coli* colonies of an 18-hour-old culture were selected with a sterile wire loop and after that inoculated into 0.8% NaCl suspension in a micro-centrifuge tube, gently vortexed, and adjusted to a turbidity equivalent to 0.5 McFarland standard (Amri and Juma 2016). One hundred microliters of the standardized bacterial culture was then evenly spread on the entire surface of the MHA plates using a sterile cotton swab and allowed to dry for 10 min before placing the antibiotic discs. The plates were incubated at 37°C for 24 h and after that read according to CLSI guidelines (CLSI 2016). The list of antibiotics tested includes the following: tetracycline (30 µg), ampicillin (10 µg), sulphamethoxazole-trimethoprim (25 µg), erythromycin (15 µg), chloramphenicol (10 µg), cefuroxime (30 µg), gentamycin (10 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), colistin sulphate (10 µg), cefotaxime (30 µg), and imipenem (10 µg). Isolates that were resistant to colistin by the disc diffusion method were then tested by the broth dilution method as recommended by the CLSI (2016). CLSI guidelines (CLSI 2016) recommended clinical resistance breakpoint for colistin as greater than or equal to ≥ 2 µg/ml. The isolates were then screened for *mcr*-1, *bla*TEM, and *erm*B by PCR, as previously described by Liu et al. (2016) and Iweriebor et al. (2015) while the other resistance determinants were not profiled.

Multidrug resistance phenotype and multiple antibiotic resistance index. The isolate which showed resistance to three or more classes of the antibiotics

Table I
Isolation and identification of *Escherichia coli*.

Shop	Location of shop	Samples collected	Presumptive isolates	Positive isolates	%
A	Open-air market	58	58	28	48.28
B	Open-air market	33	31	18	58.06
C	Closed store	43	43	8	18.60
D	Closed store	42	42	14	33.33
Total		176	174	68	39.08

tested was considered to be a multidrug-resistant. The MDR patterns of the isolates were recorded according to the protocol earlier described by Ateba et al. (2008). The Multiple Antibiotic Resistance Index (MARI) was calculated using the mathematical expression: MARI = x/y, where 'x' stands for the total number of antibiotics to which resistance was observed in an individual isolate and 'y' stands for the total number of antibiotics against which an individual isolate was tested.

Results

Prevalence of *E. coli*. From the 180 pork samples randomly collected from open-air markets and closed retail stores (Shop A to D) located in Alice, South Africa, 174 presumptive isolates were obtained through preliminary screening with the selective culture medium. Among the samples collected, shop A samples had the highest number of *E. coli* isolates, followed by shop B. These shops were located in open-air market. The percentage prevalence of *E. coli* isolates from shops C and D were lower (Table I).

Molecular identification of presumptive *E. coli* isolates. A total of 68 (39.08%) of the 174 presumptive isolates were confirmed as *E. coli* (Table I). Fig. 1 below shows the gel image representation of some of the confirmed isolates.

Susceptibility patterns of *E. coli* isolates. *E. coli* isolates from retail pork displayed resistance most frequently to erythromycin (100%; 68/68), cefotaxime (95.58% 65/68), ampicillin (88.23%; 60/68), cefuroxime (88.23%; 60/68), trimethoprim-sulphamethoxazole (88.23%; 60/68), and tetracycline (60.29%; 41/68). Lower resistance was observed against imipenem (50.00%; 34/68) and colistin (33.82%; 23/68). Notably, the resistance of *E. coli* isolates to ciprofloxacin (2.94%; 2/68) and norfloxacin (1.47%; 1/68) was lower than 5%. The susceptibility patterns of the isolates obtained in this study are shown in Fig. 2. Isolates showing resistance to at least three antibiotics of different classes were classified as multidrug-resistant. All isolates showing intermediate resistance were regarded as resistant. Genetic profiling for the *mrc*-1, *bla*TEM, and *erm*B resistance genes showed positive results as some of the phenotypic resistant isolates were positive for the genes profiled, as shown in Fig. 3–5. The frequencies of the profiled resistance genes among the isolates were as follow; *mrc*-1 11/23 (47%), *bla*TEM 13/34 (38%), and *erm*B 15/68 (22%).

Multiple antibiotic resistance phenotypes and index. This study characterized the antimicrobial resistance phenotype in *E. coli* isolates from retail stores and open-air markets. It was observed that 39.70% (27/68) of the study isolates were MDR, and 92.59% (25/27) of *E. coli* MDR isolates were resistant to cefotaxime – a third-generation cephalosporin. *E. coli* MDR isolates resistance to imipenem, a carbapenem used in this study and colistin was observed. The MDR pattern (Table II) indicated that the majority of

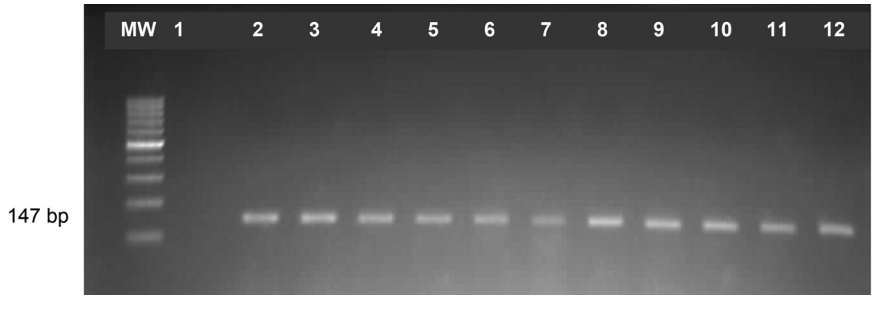

Fig. 1. Agarose gel electrophoresis for *Escherichia coli* identification.
Line M – 100 bp ladder, Line 1 – negative control, Line 2 – *E. coli* ATCC® 25922™ as a positive control, Lines 3–12 – the 147 bp PCR amplification product for *E. coli* isolates.

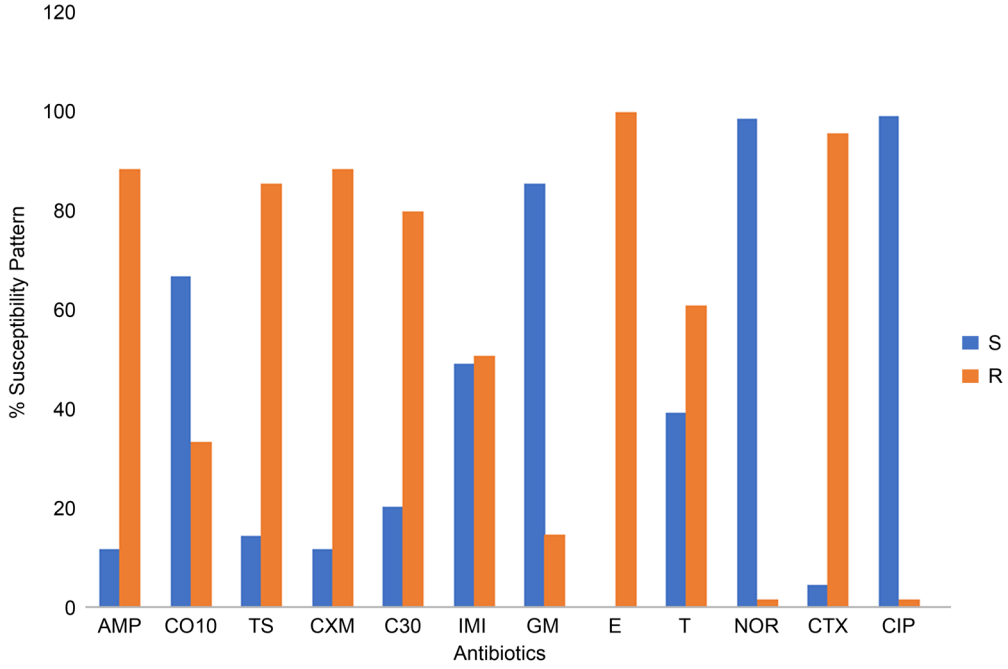

Fig. 2. The sensitivity pattern of *Escherichia coli* isolates against antibiotics.

AMP – ampicillin, CO10 – colistin sulphate, TS – trimethoprim-sulphamethoxazole, CXM – cefuroxime, E – erythromycin, C30 – chloramphenicol, IMI – imipenem, GM – gentamycin, T – tetracycline, NOR – norfloxacin, CTX – cefotaxime, CIP – ciprofloxacin, S – susceptible, R – resistant, I – intermediate

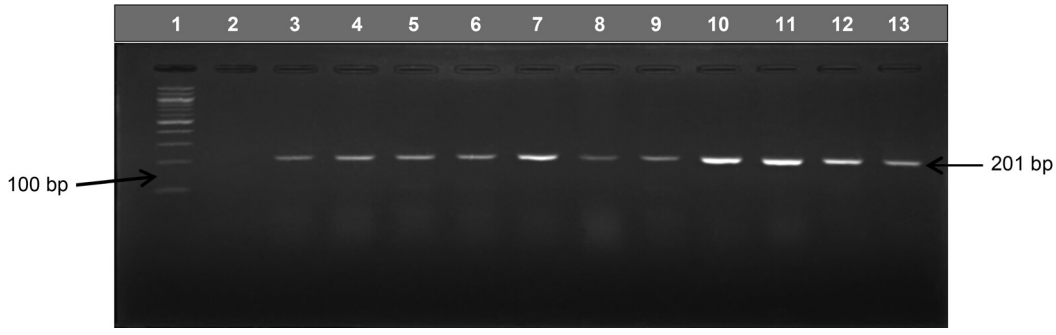

Fig. 3. Electrophoresis of *mrc-1* gene amplification among *Escherichia coli* isolates.

Line 1 – 100 bp ladder, Line 2 – negative control, Line 3–13 – positive isolates

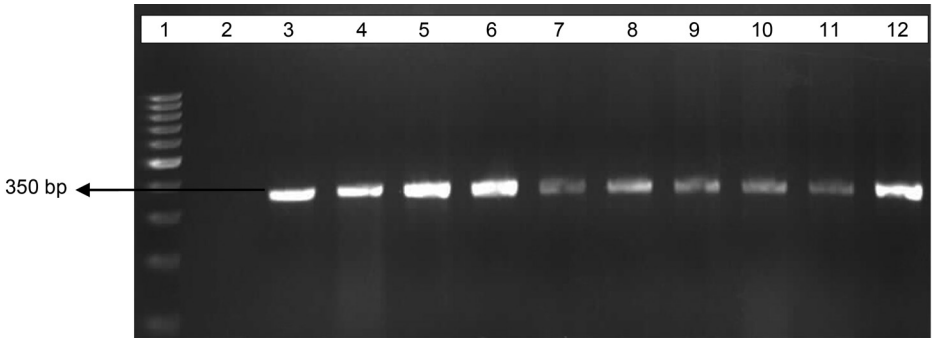

Fig. 4. Electrophoresis of the *erm*B gene amplification product (350 bp) in *Escherichia coli* isolates.

Line 1 – 100 bp DNA ladder, Line 2 – negative control, Lines 3–12 – the *erm*B-resistant *E. coli* isolates

the tested *E. coli* isolates demonstrated multiple antibiotic resistance against three to nine antibiotics. The lowest MDR rate was exhibited by 14.81% (4/27) of the isolates against three antibiotics, while 7.41% (2/27) exhibited the highest MDR rates against nine antibiotics. About 33.33% (9/27) of MDR *E. coli*

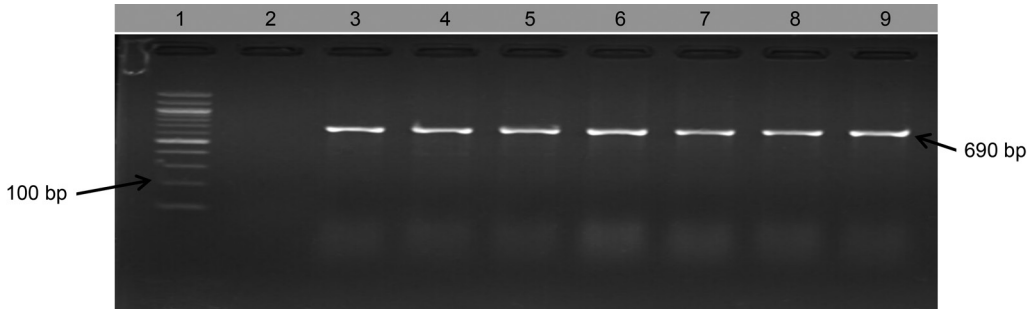

Fig. 5. Gel electrophoresis of the PCR product (690 bp) amplified with *bla*TEM primers for the detection of β-lactam-resistant *Escherichia coli* isolates.
Line 1 – DNA ladder, Line 2 – negative control, Lines 3–9 – β-lactam-resistant *E. coli* isolates

isolates were resistant to imipenem, while 29.62% (8/27) were resistant to colistin. Both imipenem resistant MDR and non-imipenem resistant MDR *E. coli* isolates were colistin-resistant (Table II and III). The MARI ranged between 0.25 and 0.75, with the average being 0.48.

Table II
Antibiotic resistance patterns and the Multiple Antibiotic Resistance Index (MARI) of the confirmed *E. coli* isolates.

MDR phenotype	Number of antimicrobials	MARI
CTX-T-E	3	0.25
CTX-E-C30	3	0.25
CTX-AMP-E	3	0.25
CTX-CXM-E	3	0.25
TS-GM-E-CO10	4	0.33
CTX-CXM-AMP-E	4	0.33
AMP-T-TS-C30-E	5	0.42
CTX-CXM-T-TS-E	5	0.42
CTX-CXM-AMP-TS-E	5	0.42
CTX-CXM-AMP-T-E	5	0.42
CTX-CXM-TS-CO10-E	5	0.42
CTX-TS-E-IMI-CIP	5	0.42
CTX-CXM-AMP-T-E-C30	6	0.50
CTX-CXM-AMP-TS-CO10-E	6	0.50
CTX-CXM-AMP-T-TS-E	6	0.50
CTX-CXM-AMP-TS-IMI-E	6	0.50
CTX-CXM-AMP-IMI-CO10-E	6	0.50
CTX-CXM-AMP-TS-IMI-E	6	0.50
CTX-CXM-AMP-TS-C30-GM	6	0.50
CTX-CXM-AMP-T-TS-C30-E	7	0.58
CTX-CXM-AMP-TS-IMI-E	6	0.50
CTX-CXM-AMP-T-TS-C30-IMI-E	8	0.66
CTX-CXM-AMP-T-TS-C30-CO10-E	8	0.66
CTX-CXM-AMP-T-TS-C30-GM-CO10	8	0.66
CTX-AMP-T-TS-E-C30-IMI-NOR	8	0.66
CTX-CXM-AMP-T-TS-C30-IMI-CO10-E	9	0.75
CTX-CXM-AMP-TS-C30-IMI-GM-CO10-E	9	0.75

MDR – indicates resistance to three or more classes of antibiotics, AMP – ampicillin, C30 – chloramphenicol, CIP – ciprofloxacin, CO10 – colistin sulphate, CTX – cefotaxime, CXM – cefuroxime, E – erythromycin, GM – gentamycin, IMI – imipenem, NOR – norfloxacin, T – tetracycline, TS – trimethoprim-sulphamethoxazole

Table III
Colistin and imipenem resistance patterns among the isolates.

Colistin- and imipenem-resistant isolates (CO10+/IMP+)	Colistin-resistant and imipenem-sensitive isolates (CO10+/IMP–)	Colistin-sensitive and imipenem-resistant isolates (CO10–/IMP+)
CTX-CXM-AMP-TS-C30-IMI-GM-CO10-E	CTX-CXM-AMP-T-TS-C30-GM-CO10	CTX-AMP-T-TS-E-C30-IMI-NOR
CTX-CXM-AMP-T-TS-C30-IMI-CO10-E	CTX-CXM-AMP-T-TS-C30-CO10-E	CTX-CXM-AMP-T-TS-E-C30-IMI-E
CTX-CXM-AMP-IMI-CO10-E	TS-GM-E-CO10	CTX-CXM-AMP-TS-IMI-E
	CTX-CXM-TS-CO10-E	CTX-CXM-AMP-TS-E
	CTX-CXM-AMP-TS-CO10-E	CTX-TS-E-IMI-CIP

AMP – ampicillin, C30 – chloramphenicol, CIP – ciprofloxacin, CO10 – colistin sulphate, CTX – cefotaxime, CXM – cefuroxime, E – erythromycin, GM – gentamycin, IMI – imipenem, NOR – norfloxacin, T – tetracycline, TS – trimethoprim-sulphamethoxazole

Discussion

The overall aim of the study was to determine MDR in *E. coli* isolated from pork sold in some retail outlets in Alice, South Africa, and depict the association of colistin and imipenem in the MDR profile. Furthermore, we determined the antimicrobial susceptibility of the isolates to other antimicrobial agents. The 39.1% prevalence of *E. coli* reported in this study is lower than in some previous studies in South Africa (Tanih et al. 2015; Jaja et al. 2020) and other countries (Xia 2010; Reddy 2017). However, a similar prevalence figure as obtained in this study was observed by Pires et al. (2020). The variation in the prevalence of *E. coli* is partly related to the method of isolation and identification. Whereas some studies identified *E. coli* with conventional bacteriological and biochemical tests, others included PCR-based molecular confirmatory tests. The presence of this bacteria in pork indicates a possible breakdown of hygiene at the different stages of the food processing and distribution chain or a lack of proper storage of the meat, as microbial contamination of food has been reported at numerous stages along the food chain distribution (Elhadi 2014). *E. coli* is a member of the microbiota of the gastrointestinal tract and can cross-contaminate meat when the content of the gastrointestinal tract bursts during slaughter. Various poor handling and unhygienic practices were observed, especially amongst retailers in the open-air market, accounting for a higher prevalence of *E. coli*. Knives for cutting meat were not washed intermittently; meats were displayed on the improperly cleaned table and were exposed to houseflies. Adzitey (2016) observed similar unhygienic practices in handling meat in Ghana and Fukuda et al. (2019) observed a relatively high proportion of flies harboring antibiotic-resistant *E. coli* and transferring resistance genes in Thailand. All these are potential sources of cross-contamination and routes of spread of infections with food-borne pathogens to humans; it also raises food safety concerns for humans, who are the ultimate consumers. Increased emergence of *E. coli* isolates with varying MDR phenotypes is a growing problem in South Africa and other developing countries. About 39% of *E. coli* isolates in this study were MDR. The MDR *E. coli* isolates showing resistance to imipenem and colistin were 33% and 29%, respectively. This implies a movement toward a pan drug resistance because colistin is the last resort antibiotic for treating infections caused by carbapenem-resistant *Enterobacteriaceae* (Liu et al. 2016). Some reports have shown colistin and carbapenem resistance genes to be located on transferable plasmids and transferred via conjugation (Fukuda et al. 2019). The emergence of resistance to antimicrobial agents such as colistin is a troubling development and public health threat, primarily as colistin is known to have a wide range of activities against the majority of the *Enterobacteriaceae* family (Liu et al. 2016; Dandachi et al. 2018). More so, besides colistin, there are few or no alternative antimicrobial agents for treating bacterial infections. Therefore, there is a need to monitor the use of colistin in the human health sector and animal husbandry (Hao et al. 2014). Currently, colistin has displaced aminoglycosides (WHO 2012; Bialvaei and Samadi Kafil 2015), and it is now considered a critically important antibiotic for human medicine despite its known high toxicity (Huang et al. 2010).

Reports have documented the occasional human use of colistin in China due to its efficacy in treating carbapenemase-producing *Enterobacteriaceae* infections (Hu et al. 2012; Zhang et al. 2015). However, in this study, the *mcr-1* resistance gene was detected in some but not all isolates with phenotypic resistance to colistin. The most probable reason for this could be that other variants of the *mcr* gene were responsible for the observed resistance or that other acquired resistance mechanisms other than the transferable *mcr* are attributable. In addition to the MDR phenotypes exhibiting co-resistance to a carbapenem and colistin, multiple resistances to other antibiotics classes were observed. Two other resistance determinants were present in the isolates phenotypically resistant to

ampicillin and erythromycin. High resistance rates among the isolates were observed against ampicillin, erythromycin, tetracycline, and trimethoprim-sulphamethoxazole. It may have resulted from their frequent use in animal husbandry, especially on the farms where the animals were raised. Sobhy et al. (2020) and Henton et al. (2011) have reported that farmers commonly use tetracycline and ampicillin as growth promoters or to prevent animal diseases. Iwu et al. (2016), in a study, carried out within the same locality where the pork was sold also reported a high level of resistance to tetracycline by *E. coli* isolated from swine. Their result was linked with farmers' reliance on tetracycline due to its availability, cost-effectiveness, and broad-spectrum activity. The habitual use of trimethoprim-sulphamethoxazole for treating infections such as respiratory infections in farm animals has been reported (Reuben and Owuna 2013) and could be linked to high resistance.

E. coli isolates exhibited the MARI of 0.25–0.75 in this study. It is also comparable with the MARI of 0.2–0.7 reported by Iwu et al. (2016) and 0.2–0.75 reported by Matyar (2012). Studies carried out by Adzitey et al. (2020) revealed a lower MARI of 0.13–0.1 in *E. coli*. The MARI observed in this study suggests the broad use of antibiotics in the swine herds from which the pork was derived, thus indicating that pork could serve as a high-risk source of multidrug-resistant organisms to humans in the study area. The occurrence of this MDR phenotype in *E. coli* is of pressing contemporary concern (Harris et al. 2015), and therefore, stresses the importance of reducing the prevalence of *E. coli* and associated resistant genes in animal husbandry through stringent regulation of antimicrobial usage in veterinary medicine.

Conclusions

In summary, the prevalence of *E. coli* isolates from retailed pork indicates fecal contamination at slaughter and processing. It calls for better hygiene practices at all stages of pork processing and highlights the importance of consumer awareness of safe pork handling and cooking. Furthermore, the association of imipenem and colistin resistance in the MDR profile is disturbing. Therefore, all unauthorized use of antibiotics, especially last resort antibiotics, should be discouraged where MDR has evolved. Therefore, the public should be educated on the perils of indiscriminate use of antimicrobial drugs. All unauthorized use of antibiotics should be discouraged. In contrast, prudent usage of antibiotics approved for veterinary use should be adopted to stem the rising trend of drug resistance among pathogenic bacteria of animal origin.

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BCI and OSE conceptualized the study, BCI collected and analyzed the samples, BCI and OSE analyzed the results, OSE helped in writing and critiquing the manuscript, CLO provided funding and proofread the manuscript.

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