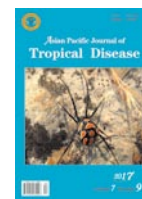


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Antibiotic resistance profiles of *Escherichia coli* O26, O145, and O157:H7 isolated from swine in the Eastern Cape Province, South AfricaChinwe Juliana Iwu^{1,2*}, Ishmael Festus Jaja³, Benson Chuku Iweriebor^{1,2}, Larry Chikwelu Obi⁴, Anthony Ifeanyi Okoh^{1,2}¹SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Private Bag X1314, Alice 5700, Eastern Cape, South Africa²Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, Eastern Cape, South Africa³Department of Livestock and Pasture Science, University of Fort Hare, Alice 5700, Eastern Cape, South Africa⁴Academic and Research Division, University of Fort Hare, Alice 5700, Eastern Cape, South Africa

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ABSTRACT

Objective: To conduct antimicrobial resistance profile of *Escherichia coli* (*E. coli*) serogroups isolated from swine that are regularly exposed to clinically important antibiotics in the farms, calculate multiple antibiotic resistance index and determine the presence of resistance determinants.

Methods: Identification of the *E. coli* isolates ($n = 169$), delineation into serogroups, and resistance genes determination were done using PCR technique. Antibiotic susceptibility testing against 16 antibiotics was done using the disk diffusion method.

Results: The susceptibility test showed that most of the isolates were highly resistant to tetracycline (long-acting counterpart), oxytetracycline and also to ampicillin (84%–100%). On the other hand, a relatively high susceptibility to norfloxacin (83%–100%), ciprofloxacin (63%–100%), gentamicin (77%–100%), and chloramphenicol (77%–100%) was observed among the isolates. The multiple antibiotic resistance indices ranged from 0.2 to 0.7 while genes encoding resistances to tetracycline, streptomycin and ampicillin were detected in 48%, 22%, and 78% of all the *E. coli* isolates, respectively.

Conclusions: Findings from this study revealed that swine from this area can be reservoirs of multiple antibiotic resistances. There is need for regular surveillance of antimicrobial usage in swine industries and the use of sub-therapeutic doses of these agents should be discouraged.

1. Introduction

Escherichia coli (*E. coli*), a member of the family Enterobacteriaceae, is found ubiquitously in the faeces of warm-blooded healthy animals[1,2]. Several pathotypes of this organism have been implicated in gastrointestinal infections in humans, with Shiga toxin-producing strains being the commonest[3,4]. Shiga toxin-producing *E. coli* (STEC) strains have been shown to be associated with various foodborne infections and outbreaks

in humans[3,5]. They have the ability to cause severe diseases including diarrhoea, haemolytic uraemic syndrome, and hemorrhagic colitis[6]. STEC are zoonotic in nature and are mostly harboured by ruminants, although they could be found in other animals such as pigs[7].

Coupled with the abuse of antibiotics by humans, their use in livestock production has been shown to play a significant role in the development of antibiotic resistance worldwide[8-10]. To meet the high demand of animal products, most modern intensive production systems use antibiotics for therapy, control and prevention of diseases and as growth promoters in animals. This practice has been shown to lead to the emergence of resistant bacteria. Therefore, it may not be strange to observe similar antibiotic resistance profiles of bacteria isolates from humans and farm animals that are regularly treated with clinically relevant antibiotics[10].

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Ethical approval was sought for and the authors ensured that all procedures performed during the studies were in accordance with the guidelines of the Ethics committee of the University of Fort Hare.

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In South Africa, studies on antibiotic resistance in isolates from food animals so far have been on *E. coli* O157 recovered from poultry, milk products and pork in northwest province[11,12], *Salmonella* from farm animals[13,14], *Enterococcus* from pigs[15] and *E. coli* O157 from cattle farms[16]. Due to the global threat to human health arising from antibiotic resistance, the need for constant surveillance studies on antibiotic resistance in farm animals becomes imperative. Therefore, the main objectives of this study were to conduct antimicrobial resistance surveillance among *E. coli* serogroups obtained from swine that are regularly exposed to β -lactams, tetracyclines, aminoglycosides, folate antagonists and fluoroquinolones, carry out multiple antibiotic resistance index (MARI) analysis, as well as profile targeted resistance determinants.

2. Materials and methods

2.1. Description of study area

The study was carried out in Nkonkobe Municipality, the second largest in the Eastern Cape Province. It is a rural municipality mostly involved in agricultural practices[14].

2.2. Ethical consideration

Ethical approval was sought for and the authors ensured that all procedures performed during the studies were in accordance with the guidelines of the Ethics Committee of the University of Fort Hare.

2.3. Bacterial strains

One hundred and sixty-nine *E. coli* isolates recovered from the two major commercial swine breeding farms between April to May 2014, within the Nkonkobe Municipality, were used for the study. One of the farms is a breeder farm which receives pigs from other farms in South Africa while the second one is an agricultural demonstration farm belonging to an agricultural institute. Samples were obtained from different animals belonging to different houses within the farm at various times to avoid duplication of animals. The isolates were obtained as follows: faecal samples which were collected on sterile swab sticks were inoculated into about 10 mL tryptic soy broth (Merck, South Africa) and incubated at 37 °C for 24 h. The resulting culture was then streaked on sorbitol MacConkey agar (Mast Group Ltd, United Kingdom) and then incubated at 37 °C for 24 h. Colourless and pink colonies were assumed to be presumptive for STEC species-O157 and non-O157, respectively [17,18].

2.4. Molecular identification of isolates

The boiling method as described by Maugeri *et al.*[19] was used to extract the genomic DNA, with slight modification as follows; a suspension of pure colonies of the presumptive isolates and sterile, free water (Thermo Scientific, USA) was made in a DNase/RNase-free Eppendorf tube. After vortexing, the suspension was heated in a

heating block (Lasec, UK) at 100 °C for 15 min. This was followed by centrifugation for 10 min at 13400 r/min and the supernatant was collected into a sterile Eppendorf tube and stored at -20 °C to be used for further assays.

Isolates were confirmed to be *E. coli* using PCR, targeting the *uidA* gene, with *E. coli* ATCC 25922 as the positive control. The reaction mixture comprised of 12.5 μ L 2 \times PCR master mix (Thermo Scientific, USA), 1 μ L of 10 pmol each of the forward and reverse primers (F: 5'-CTGGAAGAGGCTAGCCTGGACGAG-3') and (R: 5'-AAAATCGGCACCGTGGAGCGATC-3'), respectively, 5 μ L of DNA template and nuclease-free water to a total volume of 25 μ L. The PCR conditions for *uidA* amplification were as described elsewhere[20] with slight adjustment as follows: initial denaturation at 94 °C for 5min, which was followed by 35 cycles of 30 s denaturation at 95 °C, primer annealing at 58 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 8 min. The PCR amplicons (5 μ L) were verified on 1.5% agarose gel (Separations, South Africa) stained with ethidium bromide (0.001 μ g/mL), and electrophoresed at 100 V for 60 min along in a 0.5 \times Tris-borate-EDTA buffer, with a 100 bp ladder, and then visualized under UV transilluminator (Alliance 4.7).

The confirmed *E. coli* isolates were delineated into *E. coli* serogroups O26, O103, O111, O121, O145, and O157, commonly associated with foodborne illnesses in humans and also screened for virulence genes *stx1*, *stx2*, *eae*, *ehxA*, using PCR. The reaction mixture and conditions were the same as described above, except that the annealing temperature was adjusted to 55 °C for 45 s. Primer sequences for the detection of O26, O103, O111, and O145 serogroups were described by Perelle *et al.*[21], O157:H7 by Wang *et al.*[22] and O121 by Fratamico *et al.*[23]. The primer sequences for the virulence genes *stx1* and *stx2* were described by Franck *et al.*[24] while the primer sequences for *eae* and *ehxA* were described by Perelle *et al.*[21]. Both 50 bp and 100 bp DNA ladder (Promega, USA) were used for corresponding band sizes.

2.5. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on all *E. coli* isolates using the disk diffusion method according to the Clinical Laboratory Standard Institute guidelines[25]. About 4–5 colonies grown on nutrient agar (Merck, South Africa) at 37 °C for 18–24 h were suspended in normal saline. The cell density was adjusted to a 0.5 McFarland turbidity standard (equivalent to 1.5×10^8 cells). Using sterile cotton swabs, the suspension was evenly spread on Mueller-Hinton agar plate and allowed to dry for about 10 min. Appropriate antibiotic discs (Mast Diagnostics, UK) were dispensed on the surface of the agar using antibiotic disc dispenser (Mast Diagnostics, UK). The following antibiotics were tested: tetracycline (30 μ g), oxytetracycline (30 μ g), ampicillin (10 μ g), sulphamethoxazole/trimethoprim (25 μ g), streptomycin (10 μ g), gentamicin (10 μ g), amikacin (30 μ g), ceftazidime (30 μ g), cephalothin (30 μ g), cefotaxime (30 μ g), chloramphenicol (10 μ g), norfloxacin (10 μ g), ciprofloxacin (5 μ g), nalidixic acid (30 μ g), cefuroxime (30 μ g), imipenem (10 μ g). After incubation at 37 °C for 16–18 h, the plates were read and interpreted according

to the Clinical and Laboratory Standards Institute guidelines[25], using *E. coli* ATCC 25922 as quality control. Multidrug resistance was defined as resistance to 3 or more antibiotics from different classes[1].

2.6. Multiple antibiotic resistance (MAR) phenotypes and MARI

E. coli serogroups O26, O145 and O157:H7 obtained from the delineation were characterized for their MAR phenotypes while MARI was calculated and deduced using the formula: a/b, where a represents the number of antibiotics to which an isolate was resistant

to and b is the total number of antibiotics screened[26]. Isolates classified as intermediate by inhibition zone were considered as sensitive for the MARI. A MARI of ≥ 0.2 points was towards a high-risk environment where antibiotics were frequently used[27].

2.7. Screening for antimicrobial resistance genes

The *ampC*, *tetA* and *strA* genes encoding for resistance to ampicillin, tetracyclines, and streptomycin respectively, were investigated using gene specific primers in a monoplex PCR. The primer sequences, PCR conditions and amplicon size of the target genes are shown in Table 1.

Table 1

The primer sequences, PCR conditions and amplicon size of antibiotic resistance genes targeted among all the *E. coli* isolates.

Antimicrobial (gene)	Primer sequence(5'-3')	PCR condition	Product size	Reference
Ampicillin (<i>ampC</i>)	F-AATGGGTTTTCCGGTCTG R-GGGCAGCAAATGTGGAGCAA	5 min initial denaturation at 94 °C followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, final incubation at 72 °C for 5 min	191 bp	[28]
Tetracycline (<i>tetA</i>)	F-GGCCTCAATTCCTGACG R-AAGCAGGATGTAGCCTGTGC	5 min initial denaturation at 94 °C followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, final incubation at 72 °C for 5 min	372 bp	[29]
Streptomycin (<i>strA</i>)	F-CCAATCGCAGATAGAAGGC R-CTTGGTGATAACGGCAATTC	5 min initial denaturation at 94 °C followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min, final incubation at 72 °C for 5 min	548 bp	[30]

3. Results

3.1. *E. coli* serogroups and virulence genes

From the 169 confirmed *E. coli* isolates, the following serogroups were identified: *E. coli* O26 (20.7%, 35/169), *E. coli* O145 (8.3%, 14/169) and *E. coli* O157:H7 (25.4%, 43/169). Virulence gene detected was only *stx2* in 13% (22/169) of total *E. coli* isolates, with 7 isolates out of these belonging to the *E. coli* O26 serogroup. No other virulence gene was identified.

3.2. Antimicrobial susceptibility profile

Figures 1–4 show the antibiotic susceptibility pattern of *E. coli* O26, O145, O157: H7 and other *E. coli* isolates which do not belong to any of the screened serogroups, respectively.

From the antibiotic resistance profile, *E. coli* O26 strains showed a high percentage of resistance to tetracycline (100%), and oxytetracycline (100%) and ampicillin (91.4%). The percentage of resistance to streptomycin was 57%, while that of the first generation cephalosporin, cephalothin was 25.7%. The lowest percentage of resistance by *E. coli* O26 was observed against ciprofloxacin (0%) and nalidixic acid (5.7%). Serogroups O145 and O157:H7 also exhibited high prevalence of resistance against tetracycline, oxytetracycline, and ampicillin. Unlike O26 and O157:H7, *E. coli* O145 demonstrated a 100% resistance to streptomycin. Serogroup O157:H7 showed a relatively high percentage of resistance to cephalothin (42%), cefuroxime (56%) and ceftazidime (35%) which were first, second and third generation cephalosporins, respectively. All the *E. coli* isolates isolated in this study showed resistance to more than three classes of antibiotics, hence categorized as multi-

drug resistant strains. These strains were highly sensitive to the fluoroquinolones such as ciprofloxacin and norfloxacin.

3.3. MAR phenotypes and MARI

The MAR phenotypes and MARI of all the *E. coli* serogroups are shown in Tables 2–4. All the isolates were resistant to at least four antibiotics. The predominant MAR phenotype was T-AMP-S-OT, which occurred in 14%, 85%, and 53% in *E. coli* O26, O145, and O157:H7, respectively. The average MARI recorded for the serogroups were 0.35, 0.33 and 0.43 for *E. coli* O26, O145 and O157:H7, respectively.

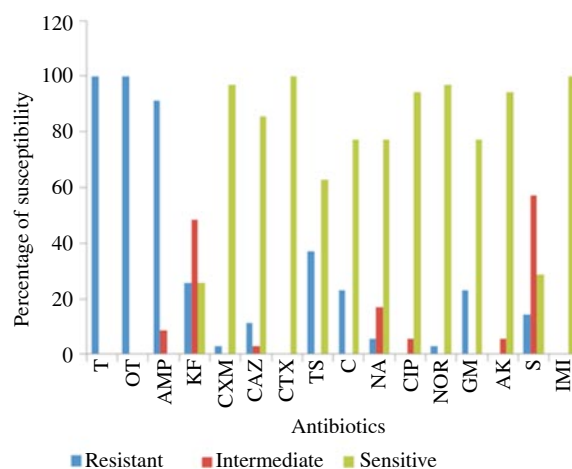


Figure 1. Antibiotic susceptibility pattern of *E. coli* O26 (n = 35).

T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

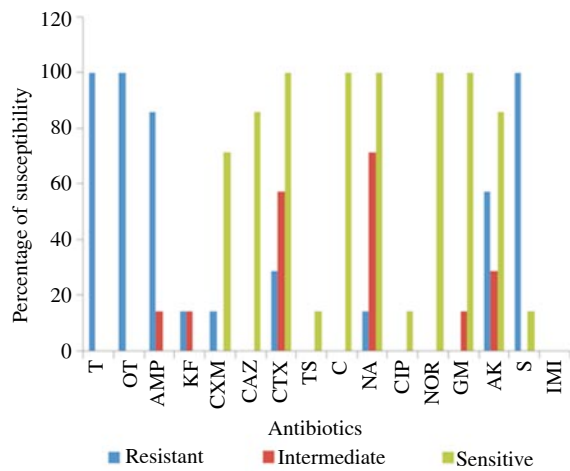


Figure 2. Antibiotic susceptibility pattern of *E. coli* O145 ($n = 14$). T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

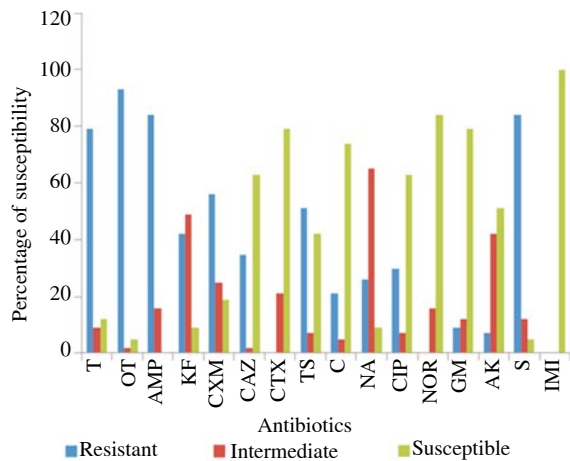


Figure 3. Antibiotic susceptibility pattern of *E. coli* O157:H7 ($n = 43$). T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

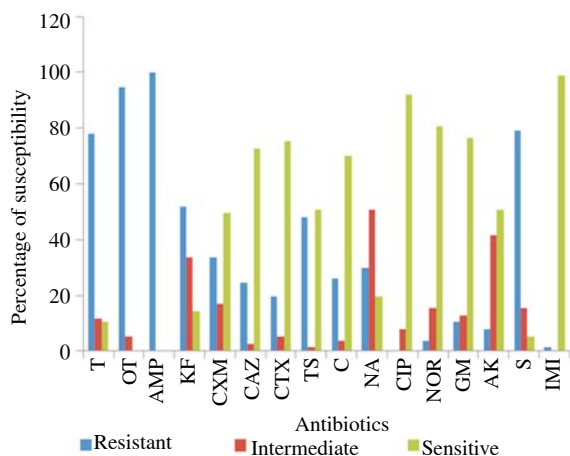


Figure 4. Antibiotic susceptibility pattern of other *E. coli* isolates ($n = 77$). T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

Table 2

MAR pattern and MARI of *E. coli* O26 isolates.

MAR pattern	No. of antibiotics	MARI
T-OT-AMP-T/S-NA-GM	6	0.38
T-GM-OT-AMP-KF-CXM- C-NOR	8	0.50
T-OT-T/S	3	0.19
T-OT-AMP-KF-C	5	0.31
T-OT-AMP-KF-C-GM	6	0.38
T-OT-AMP-T/S	4	0.25
T-OT-AMP-T/S-NA-GM	6	0.38
T-OT-AMP-T/S	4	0.25
T-OT -AMP- KF-CXM-C-NOR-GM	8	0.50
T-OT-AMP-T/S	4	0.25
OT-AMP-T/S	3	0.19
T-OT-AMP-KF-C	5	0.31
T-OT-AMP-T/S-NA-GM	6	0.38
T-OT -T/S	3	0.19
T-OT-AMP-CAZ-T/S-C-S	7	0.44
T-OT-AMP-KF-CXM-C-NOR-GM	8	0.50
T-OT-AMP	3	0.19
T-OT-AMP-T/S	4	0.25
T-OT-AMP	3	0.19
T-OT-AMP-CXM-T/S	5	0.31
T-OT-AMP	3	0.19
T-OT-AMP-KF-C-GM	6	0.38
T-OT-AMP-CAZ-T/S-C-S	7	0.44
T-OT-AMP-KF-C	5	0.31
T-OT-AMP-T/S-NA-GM	6	0.38
T-OT-AMP-T/S-NA-GM	6	0.38
T-OT-AMP-CAZ-T/S-C-S	7	0.44
T-OT-AMP-KF-CXM-C-GM	7	0.44
T-OT-AMP-KF-C-GM	6	0.38
T-OT-AMP-T/S	5	0.31
T-OT-AMP-KF-C	5	0.31
T-OT-AMP-T/S	4	0.25
T-OT-AMP-KF-C-GM	6	0.38
T OT-AMP-KF-C-GM	6	0.38
T-OT-AMP-C	4	0.25

T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

Table 3

Antibiotic resistance pattern and MARI of *E. coli* O145 isolates.

MAR pattern	No. of antibiotics	MARI
T-OT-AMP-CTX-AK-S	6	0.38
T-OT-AMP-S	4	0.25
T-OT-AMP-AK-S	5	0.31
T-OT-AMP-S	4	0.25
T-OT-AMP-S	4	0.25
T-OT-AMP-KF-CTX-S	5	0.31
T-OT-AMP-S	4	0.25
T-OT-NA-S	4	0.25
T-OT-AMP-AK-S	5	0.31
T-OT-AMP-S	4	0.25
T-OT-AMP-AK-S	5	0.38
T-OT-AMP-NA	5	0.31
T-OT-AMP-CTX-AK-S	6	0.38
T-OT-S	3	0.19

T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Ceftazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

Table 4Antibiotic resistance pattern and MARI of *E. coli* O157:H7 isolates.

Resistance pattern	No. of antibiotics	MARI
OT-AMP-KF-T/S-NA-CIP	6	0.38
OT-AMP-CXM-CAZ-AK	5	0.31
OT-AMP-KF-CAZ-T/S-NA-C-S	8	0.50
T-OT-AMP-KF-T/S-NA-S	7	0.44
T-AMP-KF-CXM-CTX-T/S-C	7	0.44
T-OT-AMP-KF-CAZ-T/S-S-NA-IMI	9	0.56
T-OT-AMP-CXM-CTX-T/S-S	7	0.44
T-OT-AMP-KF-CAZ-T/S-NA-C-S	9	0.56
T-AMP-KF-CXM-CTX-T/S-S	7	0.44
T-S-GM-OT-KF-AMP-T/S	7	0.44
OT-AMP-CXM-CTX-T/S-C-S	7	0.44
T-OT-AMP-CAZ-T/S-S	6	0.38
OT-AMP-CXM-CTX-S	5	0.31
CAZ-S-AMP-T/S-CTX-CXM	6	0.38
T-OT-AMP-CAZ-C-CIP-S	7	0.44
T-OT-AMP-T/S-NA-GM-S	7	0.44
T-OT-AMP-CXM-CAZ-CTX-T/S-NA-S	9	0.56
T-OT-AMP-KF-T/S-CXM-CTX-S	8	0.50
OT-AMP-KF-CXM-CAZ-CTX-T/S-C-S	9	0.56
T-OT-AMP-KF-T/S-NA	6	0.38
T-OT-AMP-KF	4	0.25
OT-AMP-KF-T/S-NA-S	6	0.38
T-OT-AMP-CXM-CTX-T/S-C-S	8	0.50
T-OT-AMP-KF-CAZ-T/S-NA-S	8	0.50
T-CAZ-S	3	0.19
T-OT-AMP-KF-CXM-CTX-T/S-C-NA-AK	10	0.63
T-OT-CAZ-S-KF-AMP-T/S-C-NA-CTX-CXM	11	0.69
T-OT-AMP-CXM-CTX-S-C-AK	8	0.50
T-OT-AMP-CXM-CTX-T/S	6	0.38
AMP-KF-CXM-CTX	4	0.25
S-CTX-CXM	3	0.19
OT-AMP-KF-CXM-CAZ-CTX-T/S-C-NA	9	0.56
OT-AMP-KF-CXM-CTX-S	6	0.38
T-OT-AMP-KF-CTX-CXM-T/S-S	8	0.50
T-OT-AMP-CXM-CTX-T/S-C-GM-AK-S	10	0.63
T-OT-AMP-CXM-CAZ-CTX-T/S-C-S	9	0.56
T-OT-AMP-KF-CTX-T/S-NA-S	8	0.50
T-OT-AMP-CTX-T/S-GM-S	7	0.44
T-CAZ-S-OT-KF-AMP-T/S-NA-CTX-CXM	10	0.63
T-OT-AMP-S	4	0.25
T-OT-AMP-S	4	0.25
T-OT-AMP-S	4	0.25
T-OT-AMP-S	4	0.25

T: Tetracycline; OT: Oxytetracycline; AMP: Ampicillin; KF: Cephalothin; CXM: Cefuroxime; CAZ: Cefazidime; CTX: Cefotaxime; TS: Sulphamethoxazole/Trimethoprim; C: Chloramphenicol; NA: Nalidixic acid; CIP: Ciprofloxacin; NOR: Norfloxacin; GM: Gentamicin; AK: Amikacin; S: Streptomycin; IMI: Imipenem.

3.4. Distribution of antimicrobial resistance genes

The following resistance genes: *tetA*, *strA*, and *ampC* were targeted for their possible involvement in tetracyclines, streptomycin, and β -lactam resistance, respectively. The frequencies of these resistance determinants are 48%, 22%, and 78% respectively in all the isolates put together.

4. Discussion

Food-producing animals including swine, harbour bacteria in their intestinal tracts, which may be pathogenic to man. These bacteria

could serve as reservoirs of resistance determinants that may spread through the food chain, reducing the efficacy of antimicrobials used in both human and veterinary medicine[31,32].

The frequency of STEC (13%) among the isolates obtained from our study is much higher than the 0.7% and 0.9% reported by Mohlatlole *et al.*[33] and Ojo *et al.*[34], respectively. On the other hand, a relatively similar finding was reported in another study in India, while other studies showed higher percentages[2,7,35] and lack of STEC[36]. As zoonotic organisms, they pose significant threat to humans.

Findings from antimicrobial resistance patterns of the isolates indicated that most strains were highly sensitive to the fluoroquinolones with percentage sensitivity of 63%–100% and 83%–100% for ciprofloxacin and norfloxacin respectively, even though danofloxacin, a member of the fluoroquinolones is used in both farms. We also observed a similar pattern in our previous study on antibiotic and virulence determinants in *E. coli* from cattle farms in the same locality, thus supporting this finding[16]. The use of quinolones in food-producing animals poses a great concern as they are medically important antibiotics with broad-spectrum activity against several infections in humans, including foodborne infections[32]. Hence, their use in livestock should be discouraged despite the susceptibility found. Resistance against the aminoglycosides was higher with streptomycin (an earlier generation), than amikacin, a newer member of the class, supporting similar reports elsewhere[1,37]. Furthermore, even though a relatively lower prevalence of resistance was observed to the three classes of cephalosporins, a higher resistance against cefotaxime (a third generation cephalosporin), among the *E. coli* O157:H7 strain was surprisingly observed. Similarly, high percentage of resistance to tetracycline and its long-acting variant oxytetracycline were found among all the isolates, in accordance with several reports in the literature[37-40]. Resistance to tetracyclines has been shown to be very common among *E. coli* isolates recovered from pigs, probably owing to high usage by farmers, their broad spectrum of activity, low toxicity profile, cost-effectiveness, and availability[37,38,41].

In this study, a lesser proportion of the isolates (0%–26%) showed resistance to chloramphenicol, which is in accordance with a report by Tadesse *et al.*[1]. A relatively moderate percentage of resistance (31%–51%) to sulphamethoxazole/trimethoprim observed in this study is almost similar to a report among swine in the United States[37]. In addition, a significant proportion of *E. coli* isolates showed multiple drug resistance, with T-AMP-S-OT being the most dominant MAR pattern. This phenotypic pattern is similar to the report in North West Province, South Africa, except ampicillin, which was absent from their report[38]. The similar MAR phenotypic pattern observed among all the isolates in this study may be attributed to either a common strain origin or similar history of antibiotic exposure[38,42]. The MARI observed in this study (0.2–0.7) is in agreement with that reported among pig in the North West Province, South Africa[38].

The rapid spread of resistance to tetracycline among bacteria may be due to the localization of *tet* gene on plasmids, transposons and integrons which could be transferred from one bacteria to another[39,43]. A prevalence of *tetA* gene (48%) which was observed among *E. coli* isolates in this study, is shown to be higher than those reported in other studies[39,40]. Since several variants of *tet* genes code for tetracycline resistance, isolates that were not positive for the *tetA* genes in this study may be harbouring other genes responsible

for the observed phenotypic resistance against tetracycline. Also, our findings in this study revealed a high occurrence of the *ampC* gene (78%) among the study isolates which is similar to our previous finding[16], but higher than other reports elsewhere[39,44]. The AmpC β -lactamase-producing *E. coli* have emerged globally[45], and these enzymes are active on penicillins, the first, second and third generation cephalosporins[46], such as cefuroxime, ceftazidime, and cefotaxime. This has a public health importance as these antibiotics are clinically relevant and may serve as reservoirs for the extended spectrum β -lactamase genes that could be transmitted to humans. The results of screening of the isolates for the genetic capability for streptomycin resistance reveal that 22% harboured *strA* gene, and this is similar to the findings of Kozak et al.[40] who reported a prevalence of 28%.

The sub-therapeutic usage of antibiotics in commercial animal farms could have enormous consequences in the management of human infections caused by resistant pathogens. Apart from the possibility of contamination of animal products by faecal materials during slaughtering and processing, the shedding of resistant organisms into the environment could ultimately lead to the transfer of resistance determinants to environmental microorganisms thus fueling the problems associated with antibiotic resistance. Furthermore, previous studies have shown that selection pressures persisting over a significant number of years could have pre-selected a population of drug-resistant strains before antibiotic use at each farm[47]. Thus, the identification of resistant isolates in the farms under study may not necessarily be the consequence of antibiotic usage in those farms, but might be related to the indiscriminate use of antimicrobial agents over an extended period. In future studies, we plan to collect isolates from environmental samples to test this hypothesis.

In this study, the phenotypic and genotypic resistance profiles of all the isolates depict a high level of multidrug antibiotic resistance, supported by the MARI in all the serogroups, which exceeded the threshold limit. Hence, the presence of antibiotic resistance genes in both potentially pathogenic and commensal bacteria calls for concern as this serves as a reservoir for transmission of these genes to other environmental bacteria. Therefore, the indiscriminate use of antibiotics as growth promoters at sub-therapeutic levels among animals should be discouraged through tight regulation by relevant authorities. The limitation of this study is that few resistance determinants were profiled therefore necessitating further studies including a screening of more samples and targeting more organisms other than *E. coli*.

Conflict of interest statement

We declare that we have no conflict of interest.

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