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Epidemiology and antimicrobial resistance profiles of pathogenic *Escherichia coli* from commercial swine and poultry abattoirs and farms in South Africa: A One Health approach

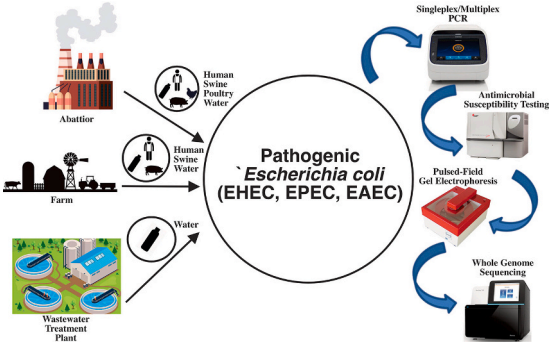
Samuel T. Ogundare [a,*], Folorunso O. Fasina [b,d], John-Paul Makumbi [a], Gerbrand A. van der Zel [e], Peter F. Geertsma [e], Marleen M. Kock [a,f], Anthony M. Smith [a,c], Marthie M. Ehlers [a,f]

[a] Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa
[b] Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa
[c] Centre for Enteric Diseases, National Institute for Communicable Diseases, Division of the National Health Laboratory Service, Johannesburg, South Africa
[d] Food and Agriculture Organisation of the United Nations, FAO Headquarters, Rome, Italy
[e] Gauteng Department of Agriculture, Rural Development and Environment, Pretoria, South Africa
[f] Department of Medical Microbiology, National Health Laboratory Service, Tshwane Academic Division, Pretoria, South Africa

HIGHLIGHTS

- First report of novel *stx2k* subtype in South Africa
- Diverse clinically relevant pathogenic *Escherichia coli* STs identified in swine, poultry, human hand swabs and run-off water
- Prevalence of EHEC was low compared to EPEC in swine and poultry sources
- Transmission of EHEC ST206 along the farm-to-fork chain
- Phylogenetic analysis showed close relationship between PEC isolates

GRAPHICAL ABSTRACT



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ABSTRACT

Pathogenic *Escherichia coli* (PEC) are important foodborne bacteria that can cause severe illness in humans. The PECs thrive within the intestines of humans as well as animals and may contaminate multiple ecosystems, including food and water, via faecal transmission. Abattoir and farm employees are at high risk of PEC exposure, which could translate to community risk through person-to-person contact. To determine the epidemiology and resistome of PECs in Gauteng and Limpopo provinces of South Africa, 198 swine faecal samples, 220 poultry cloacal swabs, 108 human hand swabs, 11 run-off water samples from abattoirs and farms were collected from four swine and five poultry commercial abattoirs and two swine farms. One effluent sample each was collected from four wastewater treatment plants (WWTP) and a tertiary hospital setting. Phenotypic and genotypic techniques were used including polymerase chain reaction, pulsed-field gel electrophoresis (PFGE) and whole

* Corresponding author at: Department of Medical Microbiology, Faculty of Health Sciences, University of Pretoria, Private Bag X323, Pretoria 0001, South Africa.
E-mail address: stogundare@gmail.com (S.T. Ogundare).

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genome sequencing (WGS). Results showed EHEC and EPEC prevalence was 4.1 % (22/542) and 20.8 % (113/542), respectively, with the O26 serogroup detected the most in PEC isolates. According to the PFGE dendrogram, isolates from poultry, human hand swabs and run-off water clustered together. Diverse virulence factors such as the novel *stx2k* subtype and *eae* genes were detected among the 36 representative PEC isolates according to WGS. The results showed that 66.7 % (24/36) of sequenced PECs presented with multi-drug resistance (MDR) to β-lactamase 13.9 % (5/36), aminoglycoside 61.1 % (22/36), tetracycline 41.7 % (15/36) and quinolones 38.9 % (14/36). No colistin nor carbapenem resistance was detected. Sequence types (STs) associated with MDR in this study were: ST752, ST189, ST206, ST10, ST48 and ST38. The findings highlight the threat of zoonotic pathogens to close human contacts and the need for enhanced surveillance to mitigate the spread of MDR foodborne PECs.

1. Introduction

Meat and meat product demands continue on an increasing trend as a result of the surged global population explosion with meat consumption projected to rise fivefold within the next decade, particularly in the low and middle-income countries (LMICs) (OECD/FAO, 2020). Consequently, this increasing demand poses significant challenges to food safety across the entire farm-to-fork value chain, increasing the risk of antimicrobial resistance and microbial contamination of food products at various stages, including slaughter, processing, packaging, storage and retail (Heredia and García, 2018). Contaminated products increase the risk of exposure to opportunistic infections in vulnerable populations (e.g., infants, the elderly or immunocompromised individuals), primarily through ingestion of zoonotic foodborne bacteria such as *Listeria monocytogenes*, *Salmonella* spp., *Enterococcus* spp., *Staphylococcus* spp. and pathogenic *Escherichia coli* (PEC) (Manyi-Loh et al., 2018).

Antimicrobial resistance (AMR) is a global threat with significant impact on public health and the economy, and has been referred to as "*the silent tsunami facing modern medicine*" (Exner et al., 2017). Failure to address AMR promptly could result in a staggering death toll by 2050, with one person dying from an AMR infection every 3 s (O'Neill, 2016). The extensive use of antimicrobials in animal husbandry has played a major role in the emergence and spread of AMR foodborne pathogens, particularly the multidrug-resistant (MDR) strains that exhibit resistance to critical antimicrobials such as extended-spectrum beta-lactamases (ESBLs), carbapenems, colistin and tigecycline (He et al., 2019; Kantele et al., 2020; Aworh et al., 2021). Studies have shown that abattoirs and farms in high-income and LMICs including South Africa serve as hotspots for the transmission of AMR foodborne pathogens to individuals in close contacts, such as abattoir workers, farmers and farmworkers as well as to the surrounding environment, which can escalate into community-wide risk through person-to-person contact (Manyi-Loh et al., 2018; Abdalla et al., 2021; Aworh et al., 2021).

Annually, *E. coli* and it's pathogenic pathotypes are estimated to cause over 2 million deaths globally (Jang et al., 2017). Notable among these PECs are the enteropathogenic *E. coli* (EPEC), the enterotoxigenic *E. coli* (ETEC) and the Shiga toxin–producing *E. coli* (STEC) (Florez-Cuadrado et al., 2018). Enterohaemorrhagic *E. coli* (EHEC), a subset of STEC, along with EPEC and ETEC, are zoonotic pathogens frequently associated with foodborne outbreaks and severe diarrhoea in LMICs (Johura et al., 2017). Typical EHEC can cause severe infections in humans and is considered the major aetiologic agent for the haemolytic-uraemic syndrome (HUS) and haemorrhagic colitis (HC) (Espinosa et al., 2018). The EPEC and ETEC on the other hand are respectively linked with infantile and travellers' diarrhoea (Cabrera-Sosa and Ochoa, 2020; Kantele et al., 2020). Due to their zoonotic potential, PECs are effective indicator for monitoring and tracking the spread of AMR both in pathogenic and non-pathogenic commensals across different environments and host species (Singh et al., 2018).

Enterohaemorrhagic *E. coli* infection is aided by a major virulence factor, the Shiga toxin (Stx), which is divided into two subfamilies (Stx1, Stx2) and subtypes (*stx1a*, *stx1c*, *stx1d*, *stx1e*, *stx2a*, *stx2b*, *stx2c*, *stx2d*, *stx2e*, *stx2f*, *stx2g*, *stx2h*, *stx2i*, *stx2j*, *stx2k* and *stx2l*) (Sánchez et al., 2021; Shen et al., 2022). The EPEC pathotype harbour the intimin (*eae*) gene, while the ETEC pathotypes harbour the plasmid-encoded heat-labile LT (*eltB*) and/or heat-stable STa/STb (*estA/estB*) toxins (Hazen et al., 2017; Shen et al., 2022). The emergence of hybrid PEC pathotypes possessing virulence factor combinations such as the EHEC/EPEC (*stx/eae*), EHEC/ETEC (*stx/eltB/estA/estB*) and EPEC/ETEC (eae/*eltB/estA/estB*) gene combinations have been reported as a major concern in South Africa and around the world (Hazen et al., 2017; Santos et al., 2020).

In South Africa, a large proportion of antimicrobial consumption in food animals is used in swine and poultry production which could drive the emergence of MDR PECs (Moyane et al., 2013). To effectively monitor and control the spread of MDR PECs in food producing animals, it is crucial to establish surveillance systems that track the emergence and prevalence of resistant PEC strains (Zhang et al., 2017). The AMR, virulence profiles and mobilomes of PEC in animal sources and the threat posed by faulty processing at abattoirs and farms in South Africa are poorly understood, necessitating this study. This study aimed to follow a One Health approach to determine the virulence profiles and AMR genes of zoonotic PEC with a focus on foodborne EHEC isolated from close human contacts, poultry, swine and environmental water samples collected from abattoirs and farms in South African provinces of Gauteng and Limpopo.

2. Materials and methods

The study was conducted in commercial poultry and swine abattoirs and farms in Gauteng and Limpopo provinces of South Africa (Fig. 1). Fig. 1 illustrates the locations of the sampled sites and their sources of supply. These two provinces have a combined swine and poultry populations of 457,562 and 22,497,719, respectively, with Gauteng province contributing >2/3 of this population (STATS SA, 2020). This study's sample size was determined using a simple random sampling method and the formula (Thrusfield, 2013):

$$n = \frac{1.96^2 P_{exp}\,(1\text{–}Pexp)}{d2}$$

where P_{exp} denotes the projected prevalence (50 %) and d denotes the desired absolute precision (5 %). The required minimum sample size was 384. In total, this study collected five hundred and forty-two (542) cloacal swabs, faecal samples, human hand swabs and run-off water samples from abattoirs, farms, wastewater treatment plants (WWTPs) and a hospital setting.

2.1. Ethical approvals

Ethical approvals for the study were obtained from the University of Pretoria Animal Ethics Committee (H012-18) and the Faculty of Health Sciences Research Ethics Committee (485/2018), Gauteng Province National Health Research Database (GP_201903_032), South African Department of Agriculture, Forestry and Fisheries Section 20 [12/11/1/1/19 (1314)] and the City of Tshwane Utility Services Department (W9/1/2/1). Permission for sampling in abattoirs and farms was obtained from the Department of Veterinary Public Health of the Gauteng Department of Agriculture and Rural Development (GDARD). Consent

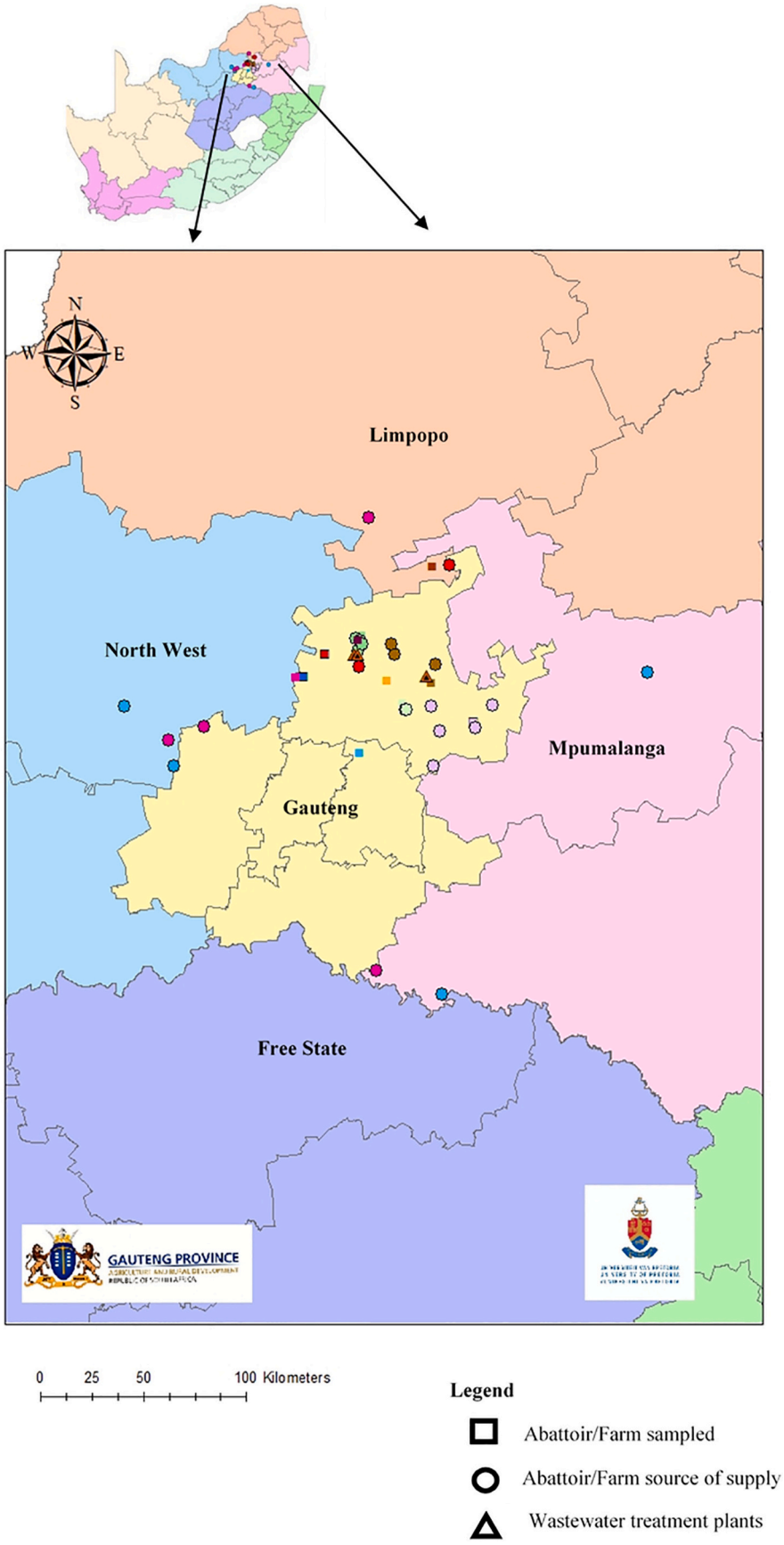

Fig. 1. Map showing locations and sources of supplies for sampled abattoirs, farms and wastewater treatment plants in Gauteng and Limpopo provinces of South Africa. Each abattoir and farm sampled appeared in similar color to their source of supply.

forms were signed by all abattoir/farm owners and workers confirming their understanding and acceptance of the study objectives and their voluntary participation.

2.2. Sample collection

Between May 2019 and August 2020, poultry (n = 220), swine (n = 198), human hand swabs (n = 108) and abattoir/farms run-off water (n = 11) were collected from poultry abattoirs (n = 5), swine abattoirs (n = 4) and medium scale commercial swine farms (n = 2) across Gauteng and Limpopo provinces (Fig. 1). In addition, effluent samples were obtained from WWTPs (n = 4) and a hospital setting (n = 1). Cloacal swabs from poultry and human hand swab samples were collected using sterile cotton swabs in Amies transport medium (without charcoal) (Oxoid Ltd., United Kingdom) while faecal samples from swine were collected in sterile (screw-cap) containers (Greiner Bio-One, Germany). Eight litres of run-off water samples were collected aseptically in sterile glass bottles from each abattoir, farms and WWTPs visited. All samples were labelled and transported on ice packs in iceboxes to the laboratory for processing within three hours.

2.3. Sample processing and isolation of pathogenic Escherichia coli

All cloacal swabs from poultry, faecal samples from swine (0.2 g of soft stool) and human hand swab samples were homogenised in 10 mL of brain heart infusion (BHI) broth (Lab M Limited, UK) and incubated (Vacutec, South Africa) at 37 °C overnight in an orbital shaker (Si500, Bibby Scientific Group, UK) at 230 rotation per minutes (rpm). One hundred microliters (100 μL) of enrichment suspension were spread on CHROMagar™ STEC (CHROMagar Microbiology, France) and incubated overnight at 37 °C for 18 h to 24 h (Vacutec, South Africa). Similarly, 100 mL of run-off water samples were filtered through 0.45 μM Millipore™ membranes filter (Merck, Germany) repeatedly until the sample was exhausted. The membrane filters were aseptically placed directly onto the CHROMagar™ STEC (CHROMagar Microbiology, France) and incubated (Vacutec, South Africa) at 37 °C for 18 h to 24 h. Following incubation, cultures were examined for bacterial growth and colony morphology. Colonies displaying a mauve color were considered presumptive PEC, following the manufacturer's manual. A minimum of three mauve and maximum of five colonies per plate were randomly chosen, subcultured in 2 mL of BHI broth (Lab M Limited, UK) and incubated (Vacutec, South Africa) at 37 °C for 18 h to 24 h.

2.4. Molecular identification of pathogenic Escherichia coli

Total genomic DNA was extracted from presumptive PEC isolates subculture in 2 mL BHI broth (Lab M Limited, UK) at 37 °C for 18 h to 24 h using the ZR Fungal/Bacterial DNA Miniprep™ commercial kit (Zymo Research, USA) according to the manufacturer's instructions. Conventional multiplex polymerase chain reaction (M-PCR) assays targeting the PEC serogroups (O165, O103, O111, O157, O26, O121 and O145) and virulence genes (*eae*, *stx1, stx2*) (Iguchi Atsushi et al., 2015) were used in this study to screen all presumptive PEC isolates. All the primers used in this study were synthesised by Inqaba Biotechnical Industries (Pty) Ltd., South Africa as shown in Supplementary Table 1. Isolates which were positive for at least one gene were screened for the haemolysin (*hlyA*) gene by a singleplex PCR assay (Paton and Paton, 1998). The assay conditions were optimized using sterile nuclease-free water (Qiagen, Germany) as negative control and a positive control strain obtained from a previously characterised EHEC O157:H7 strain that encode for O157, *eae*, *stx1*, *stx2* and *hlyA* genes (Smith et al., 2011; Tau et al., 2012). Supplementary Table 2 and Supplementary Table 3 shows the multiplex and singleplex PCR reaction's composition and amplification used in this study. All confirmed PEC isolates were stored in 50 % sterile glycerol (Merck, Germany) for further analysis.

2.5. Phenotypic detection and antimicrobial susceptibility testing of pathogenic Escherichia coli isolates

All positive PEC isolates were tested for extended-spectrum beta-lactamases (ESBL) production by the Double Disc Synergy Test (DDST) and carbapenemase production using the modified carbapenem inactivation method (mCIM) according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines (Appendix A and B). A rapid colistin NP test for detection of polymyxin resistance as previously described (Nordmann et al., 2016) was used to test polymyxin resistance in all PEC isolates (Appendix C). The PEC isolates that tested positive for either of the phenotypic EBSLs, carbapenemase and polymixins tests were identified and analyzed for antimicrobial susceptibility (AST) using an automated system, the WalkAway 40 plus MicroScan system (Beckman Coulter Inc., California, USA) following the manufacturer's instructions (Appendix D).

2.6. Multiplex PCR amplification of selected antimicrobial resistance genes in PEC isolates

Multiplex polymerase chain reaction assays, as previously described (Dallenne et al., 2010; Rebelo et al., 2018; Borowiak et al., 2020) were used to screen all presumptive ESBL-producing PEC isolates recovered from phenotypic testing for the detection of ESBL genes (CTX-M groups, SHV and TEM), carbapenemase genes (IMP, OXA-like) and colistin resistance genes (MCR-1 to MCR-9). See Supplementary Table 2 for the M-PCR assay reaction mixture. The assay conditions were optimized using sterile nuclease-free water (Qiagen, Germany) as negative control.

2.7. Molecular typing of PEC isolates

Genotyping of PEC isolates was performed using pulsed-field gel electrophoresis (PFGE) as previously described (Greenquist et al., 2005; CDC-PulseNet, 2017). The restriction enzyme *Xba1* (New England Biolabs, USA) was used to digest the chromosomal DNA of each of the PEC isolates and PFGE was performed using the Rotaphor system (Biometra, Germany). The resulting PFGE gel bands were analyzed with GelCompar II software (Applied Maths, Belgium) and clustered using the Dice coefficient. A dendrogram was constructed by utilizing the unweighted pair group method with arithmetic mean (UPGMA). The PEC isolates sharing ≥80 % similarities were grouped as the same Pulsotypes. Pulsotypes which included five or more isolates were classified as major, while minor pulsotypes included more than two and less than five isolates. Other isolates were classified as singletons.

2.8. Whole genome sequencing of PEC

Thirty-six representative PEC isolates were chosen for whole genome sequencing (WGS) according to the PFGE pulsotypes and AMR profiles detected. The WGS was performed by the Sequencing Core Facility of the National Institute for Communicable Diseases (NICD). Genomic DNA from fresh cultures incubated overnight in BHI broth at 37 °C for 18 h to 24 h (Vacutec, South Africa) was extracted using the ZR Fungal/Bacterial DNA Miniprep™ commercial kit (Zymo Research, USA) according to the manufacturer's instructions. The DNA concentration was quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA). The DNA libraries were prepared using the Nextera DNA Flex Library Preparation Kit (Illumina). Thereafter, PEC DNA was sequenced using Illumina NextSeq 500 platform (Illumina, San Diego, CA) next-generation sequencing with paired 2 × 150 bp paired-end sequencing runs ~80 times coverage. Analysis and typing of the paired-end reads were performed using the JEKESA bioinformatics pipeline version 1.0 (https://github.com/stanikae/jekesa).

2.9. In silico detection of virulome, resistome, serogroups, multilocus sequence typing and MGEs of PEC isolates

In this study, the virulence factors for sequenced PEC isolates were detected in silico using the service of Centre for Genomic Epidemiology (CGE) VirulenceFinder (https://cge.food.dtu.dk/services/VirulenceFinder/ accessed on 23 January 2023) and the VFanalyzer platform from Virulence Factor Database (VFDB) (http://www.mgc.ac.cn/VFs/search_VFs.htm accessed on 25 January 2023), with a threshold of >90 % for both databases (Liu et al., 2019; Malberg Tetzschner et al., 2020). The virulence genes reported are a combination of both databases. The resistance genes were annotated and identified using the ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/ accessed on 23 January 2023) with default values of 90 % identity and 60 % minimum length, respectively (Florensa et al., 2022). Phylogroups of isolates were determined using the ClermonTyper tool (http://clermontyping.iame-research.center/ accessed on 19 January 2023) (Beghain et al., 2018). MobileElementFinder (https://cge.food.dtu.dk/services/MobileElementFinder/ accessed on 23 January 2023) was used for the detection of insertion sequences (IS), transposons (Tns) and other conjugative genetic elements associated with AMR genes and virulence factors (Johansson et al., 2021). PlasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/ accessed on January 23, 2023) was used to profile the plasmids replicons and incompatibility groups, with the default thresholds of 95 % minimum identity and 60 % minimum coverage (Carattoli et al., 2014). MLSTFinder 2.0 (https://cge.food.dtu.dk/services/MLST/ accessed on 23 January 2023) using the seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) with allele sequence and profile data obtained from PubMLST.org (Jolley et al., 2018) was employed to assign sequence types (STs) and clonal complex (CC). Detection and assigning of serogroups according to the O: H antigens was performed using the SerotypeFinder 2.0 (https://cge.food.dtu.dk/services/SerotypeFinder/ accessed on 23 January 2023) (Joensen et al., 2015).

2.10. Calling single nucleotide polymorphisms and phylogenomic analyses

Phylogenetic analysis from sequenced isolates in this study was performed by calling SNPs using the CGE CSI Phylogeny 1.4 tool (https://cge.food.dtu.dk/services/CSIPhylogeny/ assessed on 16 February 2023). The assembled FASTA files were uploaded and aligned with the reference isolate YA00028237 (ERS4920555) and tree file outputs were downloaded and saved as Newick files with default threshold settings. Annotation, visualisation and management of tree files were performed with the aid of the interactive Tree of Life tool—iTOL v6 (https://itol.embl.de/) (Letunic and Bork, 2021).

3. Results

Among the 542 samples collected for this study, 701 presumptive PEC mauve colonies were randomly selected for screening from samples which showed bacterial growth on CHROMagar™ STEC (CHROMagar Microbiology, France) plates (section 2.3 above) of which: 45.3 % (318/701) were obtained from poultry samples, 44.7 % (313/701) from swine samples, 5.4 % (38/701) from human hand swabs and 4.6 % (32/701) from run-off water samples collected from abattoir and farm run-off.

3.1. Detection of PEC isolates from swine, poultry, hand swabs and run-off water

The M-PCR assay showed that 20.3 % (142/701) of the presumptive isolates were PEC with a prevalence of 16.4 % (89/542) detected in the total samples collected for this study (Supplementary Table 4). The distribution of recovered PEC isolates was as follows: 45.1 % (64/142) from poultry samples, 41.5 % (59/142) from swine samples, 6.3 % (9/142) from human hand swabs collected from abattoir workers and 7 % (10/142) from environmental/run-off water samples collected from abattoirs and farms (Table 1). Although this study aimed to detect EHEC, the recovery of EPEC isolates among selected mauve colonies on CHROMagar™ STEC (CHROMagar Microbiology, France) was an interesting finding that warranted further investigation. In this study, no PEC isolate was detected in hand swabs collected from farm workers and effluents from WWTPs and hospital setting. Overall, 4.1 % (22/542) of PEC isolates were EHEC carrying the *stx2* gene. Findings in this study showed that EHEC was significantly detected in swine isolates ($P < 0.0001$). Five EHEC isolates 22.7 % (5/22) jointly carried the *stx2* and enterohaemolysin *hlyA* genes while one isolate carried the *stx2*, *hlyA* and *eae* genes (Table 1). None of the PEC isolates recovered were positive for the *stx1* gene according to the M-PCR assay except the positive control isolate. The EPEC isolates [20.8 % (113/542)] in this study carried the *eae* gene and were detected in swine, poultry, human hand swabs and run-off water isolates recovered from abattoirs and farms. The *eae* and *hlyA* genes were detected concurrently in 17.7 % (20/113) of the EPEC isolates (Table 1). Findings showed the prevalence of EHEC [3.9 % (21/542)] and EPEC [15.5 % (84/542)] in Gauteng province was higher than the prevalence of EHEC [0.2 % (1/542)] and EPEC [5.4 % (29/542)] in Limpopo province (Table 1). The O26 serogroup was the most frequently detected in this study, accounting for 21.8 % (31/142) of the isolates. However, seven PEC isolates were assigned to serogroups but did not exhibit any PEC virulence genes when analyzed with M-PCR (Table 1). Serogroups O111 and O165 were not detected by the M-PCR assay in this study.

3.2. Genetic relatedness of the PEC isolates using pulsed-field gel electrophoresis

A total of 78.9 % (112/142) isolates were typable with PFGE using *Xbal* restriction enzyme. Isolates that were untypable were stored for future studies. The dendrogram revealed the genetically diverse nature of the PEC isolates with five major (A–E) and 18 minor pulsotypes including 24 singletons (Fig. 2). Isolates from different sites did not cluster in the same pulsotype. One minor pulsotype, F clustered 3 isolates from poultry, human hand swab and run-off water detected in the same site (Fig. 2).

3.3. Phenotypic antimicrobial susceptibility testing

The evidence of ESBL-producing PEC isolates was detected using the DDST. A total of 7/142 presumptive ESBL-producing swine EHEC (1/7), poultry EPEC (4/7) and run-off water (2/7) isolates were identified. No carbapenemase-producing nor colistin-resistant PEC isolates were reported after both mCIM and rapid colistin NP test were performed on all isolates. According to WalkAway 40 plus MicroScan system (Beckman Coulter Inc., California, USA), six (6/7) presumptive ESBL-producing PEC isolates showed resistance to cephalothin (MIC ≥16 μg/mL) while two isolates 2/7 each were resistant to gentamicin (MIC ≥8 μg/mL),

Table 1
Distribution of serogroups and virulence genes among PEC isolates.

Variables	Swine (n)	Poultry (n)	Hand Swab (n)	Environment (n)
Serogroup	O26 [29], O121 [1], O145 [5], O157 [1]	O103 [7], O145 [1]	O121 [3]	O26 [2], O103 [2]
Virulence genes	*stx2* [16], *stx2* + *hlyA* [5], *stx2* + *eae* + *hlyA* [1], *eae* [15], *eae* + *hlyA* [19], *hlyA* [1], N/A [2]	*eae* [64]	*eae* [6], N/A [3]	*eae* [9], *eae* + *hlyA* [1]
Province of collection	Gauteng [28], Limpopo [31]	Gauteng [64]	Gauteng [9]	Gauteng [8], Limpopo [2]
Prevalence (%)	41.5 % (59/142)	45.1 % (64/142)	6.3 % (9/142)	7 % (10/142)

N/A: PEC serogroup detected with negative virulence genes.
Superscript: number of times PEC serogroup/virulence gene was detected.

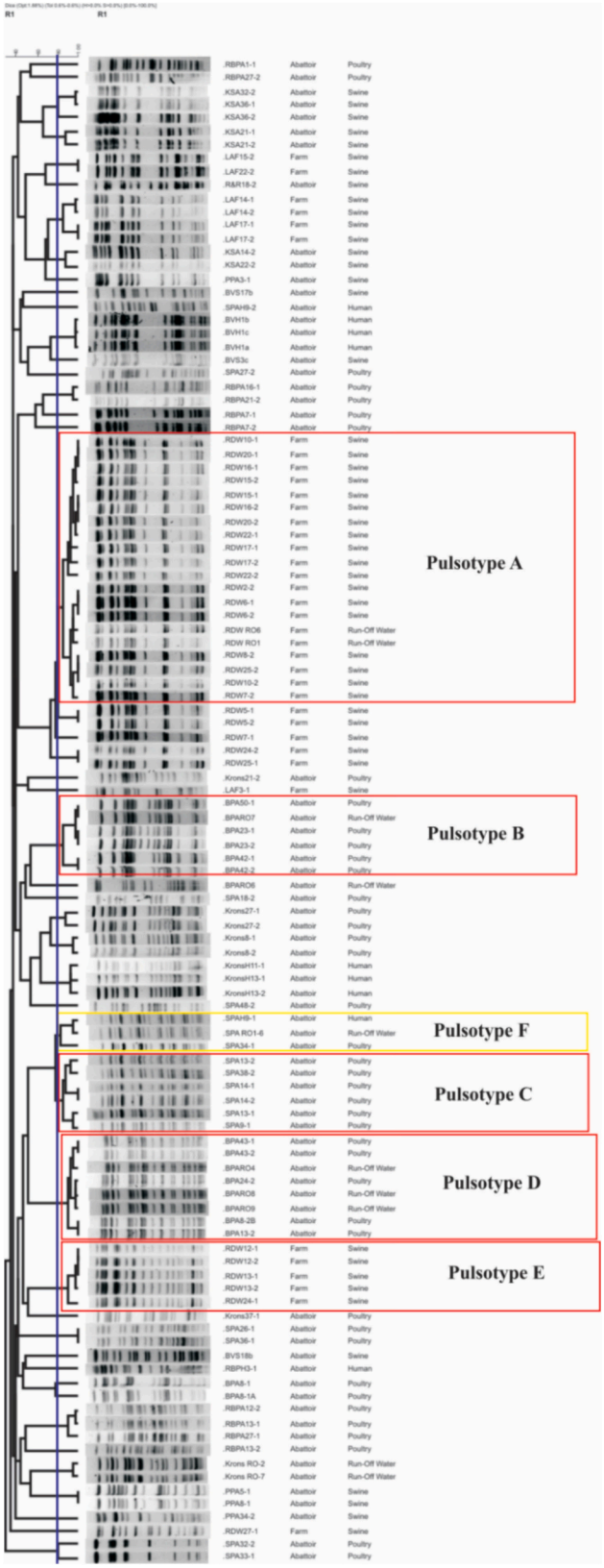

Fig. 2. The dendrogram of PFGE analysis showing genetic diversity among PEC isolates constructed from the banding patterns using the Dice coefficient and UPGMA. The dendrogram reveals five major pulsotypes and a minor pulsotype at a cut-off value of ≥80 % shown by the blue line.

fosfomycin (MIC >64 μg/mL), nitrofurantoin (MIC ≥64 μg/mL) and trimethoprim-sulfamethoxazole (MIC >2/38 μg/mL) (Supplementary Fig. 1). Resistance to 10 tested antimicrobials was observed in one EHEC swine isolate RSA-18-2 including cephalosporin antimicrobials such as ampicillin (MIC >16 μg/mL), aztreonam (MIC >8 μg/mL), cefotaxime (MIC >16 μg/mL), ceftazidime (MIC ≤1 μg/mL), cefuroxime (MIC >16 μg/mL), cefepime (MIC >8 μg/mL) and ampicillin-sulbactam (MIC >16/8 μg/mL). One EPEC environmental run-off isolate BPA-RO6 also showed resistance to eight tested antimicrobials including ciprofloxacin (MIC ≥2 μg/mL), norfloxacin (MIC ≥8 μg/mL) and tobramycin (MIC ≥8 μg/mL) (Supplementary Fig. 1).

3.4. Molecular detection of the antimicrobial resistance profiles of the PEC isolates

Multiplex PCR assays performed on the PEC isolates detected ESBL genes in 4.9 % (7/142) of the tested isolates. One swine EHEC isolate RSA-18-2 that showed phenotypic resistance to cephalosporin antimicrobials was positive for the CTX-M gp 9 gene while two swine, one poultry and three human hand swab isolates harboured the TEM gene. None of the PEC isolates revealed the presence of the ESBLs (SHV), carbapenem (IMP, OXA-like) or colistin (MCR) resistance genes according to the M-PCR assays.

3.5. Virulence factor profiles and classification of PEC isolates

Virulence factors detected with WGS on the 36 representative PEC isolates enabled the classification of isolates into three main pathotypes: (i) with EPEC the most dominant pathotype [72.2 % (26/36)] followed by (ii) EHEC [19.4 % (7/36)] and (iii) enteroaggregative *E. coli* (EAEC) [5.6 % (2/36)] (Fig. 3).

Identified virulence factors were classified into four primary functional types: (i) toxins, (ii) adherence/colonisation factors, (iii) fitness/iron uptake and (iv) invasins/non-LEE/LEE effectors with the EPEC isolates harbouring the highest prevalence of virulence factors (Fig. 3). All the PEC [100 % (36/36)] isolates in this study harboured the tellurite resistance (*terC*) gene, type 1 fimbrial adhesin (*fimH*) gene, major curlin subunit protein (*csgA*) gene and the invasin (*ibeBC*) genes. The EHEC isolates were all positive for the *stx* genes [100 % (7/7)]. The VFDB and CGE VirulenceFinder tools showed a discrepancy in detecting the *stx1* gene. VFDB found that 71.4 % (5/7) of EHEC isolates had both *stx1* and *stx2* genes, whereas CGE VirulenceFinder only detected the *stx2* gene. The *stx2e* and *stx2b* subtypes were identified in 57.1 % (4/7) and 28.6 % (2/7) of the EHEC isolates, respectively. In this study, one EHEC PSA-34-2 isolate harboured the novel *stx2k* subtype. The swine EHEC O157 isolate, positive for the *stx2* gene according to the M-PCR assay, did not detect either the *stx1* or *stx2* genes with WGS. The majority of the EHEC isolates [85.7 % (6/7)] were classified as EHEC/ETEC hybrid pathotypes, co-harbouring the EHEC *stx2* and ETEC *estA*/*estB* genes. Two PEC isolates that did not test positive for any of the virulence genes using the M-PCR assay were identified as EAEC isolates [5.6 % (2/36)] according to WGS. The EAEC isolates were recovered from one swine and one human hand swab sample from the same abattoir site and harboured the putative virulence gene *aaiC* chromosomal type VI secretion system and the EHEC-like plasmid-encoded *katP* gene (Fig. 3).

Variants of the intimin *eae* gene detected in EPEC isolates included: (i) the *eae*- β [61.5 % (16/26)], (ii) *eae*- ε [26.9 % (7/26)] and (iii) *eae*- γ [11.5 % (3/26)] genes. The EPEC isolates carrying the *eae*- ε variant was significantly associated with three toxin genes namely: *pic*, *hlyF* and *cvaC* ($P = 0.0081$). Among the fitness/iron uptake genes, the *sitA*, *iucC*, *iutA*, *tsh* and *iroN* genes were present in all [100 % (7/7)] of the *eae*- ε isolates whereas the *chuA* gene was detected in three [100 % (3/3)] *eae*- γ positive isolates. In addition, 100 % (7/7) of the *eae*- ε variant positive EPEC isolates were positive for the putative type I secretion outer membrane protein, the *estC* gene. Findings revealed the *eae*- ε isolates were detected in poultry, hand swabs collected from poultry abattoir workers and run-

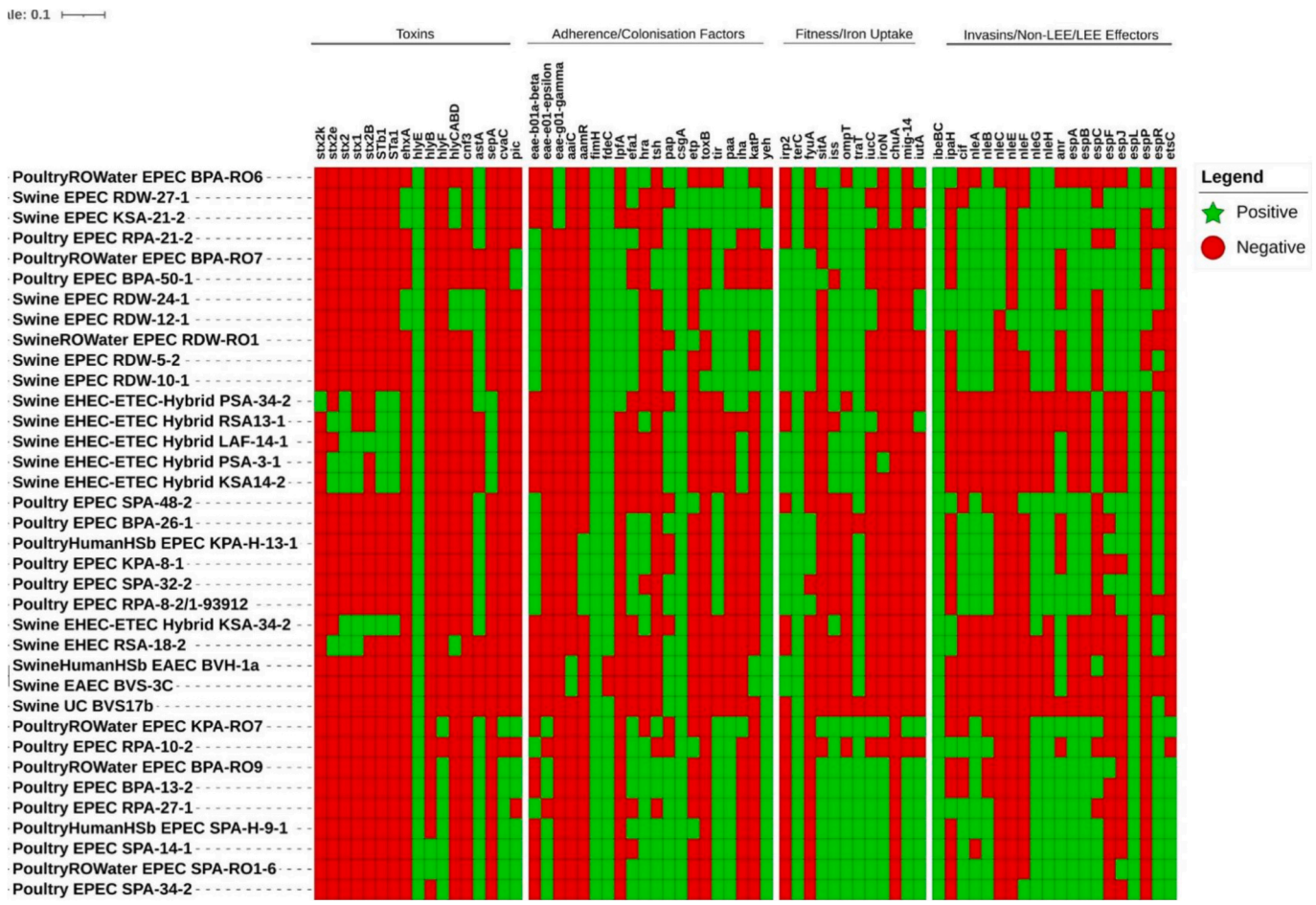

Fig. 3. Heatmap of virulence factors classified into toxins, adherence/colonisation factors, fitness/iron uptake and invasins/non-LEE/LEE effectors groups.

off water from poultry abattoirs across three different sites in Gauteng province (Fig. 3). Other virulence factors genes recovered from EPEC isolates were the EHEC-like plasmid-encoded genes: *katP* [26.9 % (7/26)], *etp* [23.1 % (6/26)], *toxB* [19.2 % (5/26)], *ehxA* [15.4 % (4/26)] and *espP* [15.4 % (4/26)]. The swine EPEC O157 isolate, RDW-27-1 harboured a complete plasmid carrying the *toxB*, *katP*, *etp*, *ehxA* and *espP* genes. The AraC negative regulator (*anr*) gene [61.1 % (22/36)] was observed in EAEC [100 % (2/2)], EHEC [71.4 % (5/7)] and EPEC

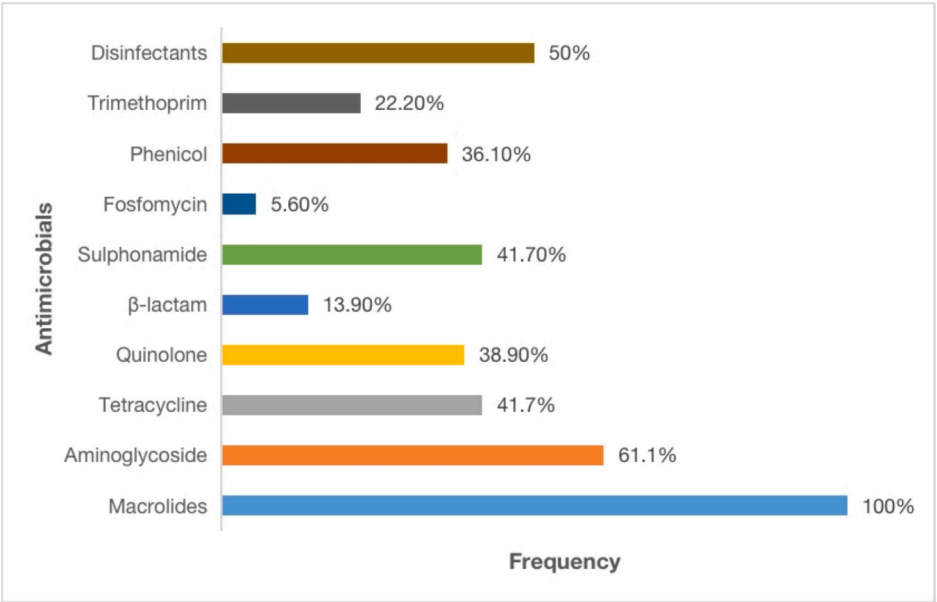

Fig. 4. Frequency of antimicrobial detected in sequenced pathogenic *Escherichia coli* representative isolates.

[57.7 % (15/26)] isolates while the increased serum survival (*iss*) gene [66.7 % (24/36)] was detected in both EPEC [73.1 % (19/26)] and EHEC [71.4 % (5/7)] isolates (Fig. 3).

3.6. Antimicrobial resistance profiles of PEC isolates determined by WGS

The PEC isolates investigated showed a variety of antimicrobials (Fig. 4). Forty-six distinct AMR genes were found among the PEC isolates analyzed (Fig. 5). In total, 33.3 % (12/36) of PEC isolates presented with less than two AMR genes whereas 66.7 % (24/36) were classed as MDR isolates with three or more AMR genes present. The β-lactam $bla_{\text{CTX-M-14}}$ gene was detected in one swine MDR EHEC isolate RSA-18-2 also co-harbouring *aph(6)-Id*, *aph(3″)-Ib*, *aadA1*, *aadA2b*, *aph(3′)-IIa*, *fosA3*, *mdf(A)*, *cmlA1*, *OqxB*, *OqxA*, *sul3* and SMR Efflux pump *qacL* gene. The $bla_{\text{TEM-1B}}$ [11.1 % (4/36)] gene was detected in two MDR swine EHEC isolates (KSA-14-2, PSA-3-1) and one each of MDR swine and human hand swab EAEC isolates (BVS-3C, BVH-1A) from three different abattoir sites (Fig. 5). One EHEC swine isolate (KSA-14-2) positive for $bla_{\text{TEM-1B}}$ also harboured the $bla_{\text{TEM-33}}$, $bla_{\text{TEM-34}}$, $bla_{\text{TEM-141}}$, $bla_{\text{TEM-206}}$, $bla_{\text{TEM-209}}$, $bla_{\text{TEM-210}}$, $bla_{\text{TEM-214}}$ and $bla_{\text{TEM-216}}$ variants (Fig. 5). One MDR EPEC isolate BPA-RO6 detected in run-off water revealed the presence of 22 AMR genes of importance including: *fosA3*, *qnrS1*, *OqxB*, *OqxA*, *sul1*, *sul2*, *sul3*, *aph(6)-Id*, *aph(3″)-Ib*, *aph(3′)-IIa*, *aadA1*, *aac(3)-IId*, *aadA5*, *mdf(A)*, *lnu(F)*, *cmlA1*, *tet(A)*, *dfrA17*, *dfrA12 sitABCD*, *qacE* and *qacL* while two other MDR EPEC isolates (SPA-H-9-1 and KPA-H-13-1) recovered from hand swabs both harboured the *aph(6)-Id*, *aph(3″)-Ib*, *mdf(A)* and *tet(A)* AMR genes (Fig. 5).

The quinolone resistance efflux pump genes *OqxB* and *OqxA* as well as plasmid-mediated quinolone resistance (PMQR) *qnrS1* and the *fosA3* gene, conferring resistance to fosfomycin were observed 33.3 % (12/36), 30.6 % (11/36), 8.3 % (3/36) and 5.6 % (2/36), respectively. Mutations in the quinolone resistance determining region (QRDR) *gyrA* (p.S83L) were identified in 11.1 % (4/36) while 8.3 % (3/36) concomitantly presented with the *gyrA*(p.S83L) and the *parC*(p.A56T) gene mutations which are likely to play a significant role in high-level fluoroquinolone resistance. Additionally, operons conferring resistance to the bactericidal effects of hydrogen peroxide *sitABCD* [27.8 % (10/36)], small multidrug resistance (SMR) efflux pump *qacL* [33.3 % (12/36)] and *qacE* efflux genes [13.9 % (5/36)] that increases tolerance of PEC isolates to antiseptics and disinfectants including quaternary ammonium compounds (QACs) were detected (Fig. 5).

3.7. Genetic characterisation and phylogenetic analysis of PEC isolates

The genome size of the 36 PEC isolates ranged from 4.7 Mbp to 5.7 Mbp with a genomic content of 50.05 to 50.82 and a minimum contig length (N50) ranging from 43 kbp to 170 kbp (Supplementary Table 5).

The in silico MLST results revealed a total of 16 distinct STs, indicating genetic diversity among the PEC strains (Fig. 5; Table 2). The PEC STs associated with MDR were ST752, ST189, ST206, ST10, ST48 and ST38. Three EHEC strains [8.3 % (3/36)] were assigned to MDR ST206, while EPEC strains were predominantly assigned to MDR ST752 and ST189 with a prevalence of 25 % (9/36) and 16.7 % (6/36), respectively (Fig. 5; Table 2). While the ST206 were recovered from swine isolates, the ST752 and ST189 were mainly recovered from poultry, environmental run-off water samples from poultry facilities and hand swabs taken from poultry abattoir workers (Fig. 5; Table 2). The majority of the MDR EPEC ST752 strains [77.8 % (7/9)] and ST189 [33.3 % (2/6)] strains belonged to the newly reported EPEC non-classical O123/186 and O80 serogroups carrying H40 and H26, respectively. Similarly, the MDR EHEC ST206 strains all belonged to serogroup O141 with an untypable H antigen (Table 2). A strain of EHEC assigned to ST10 and

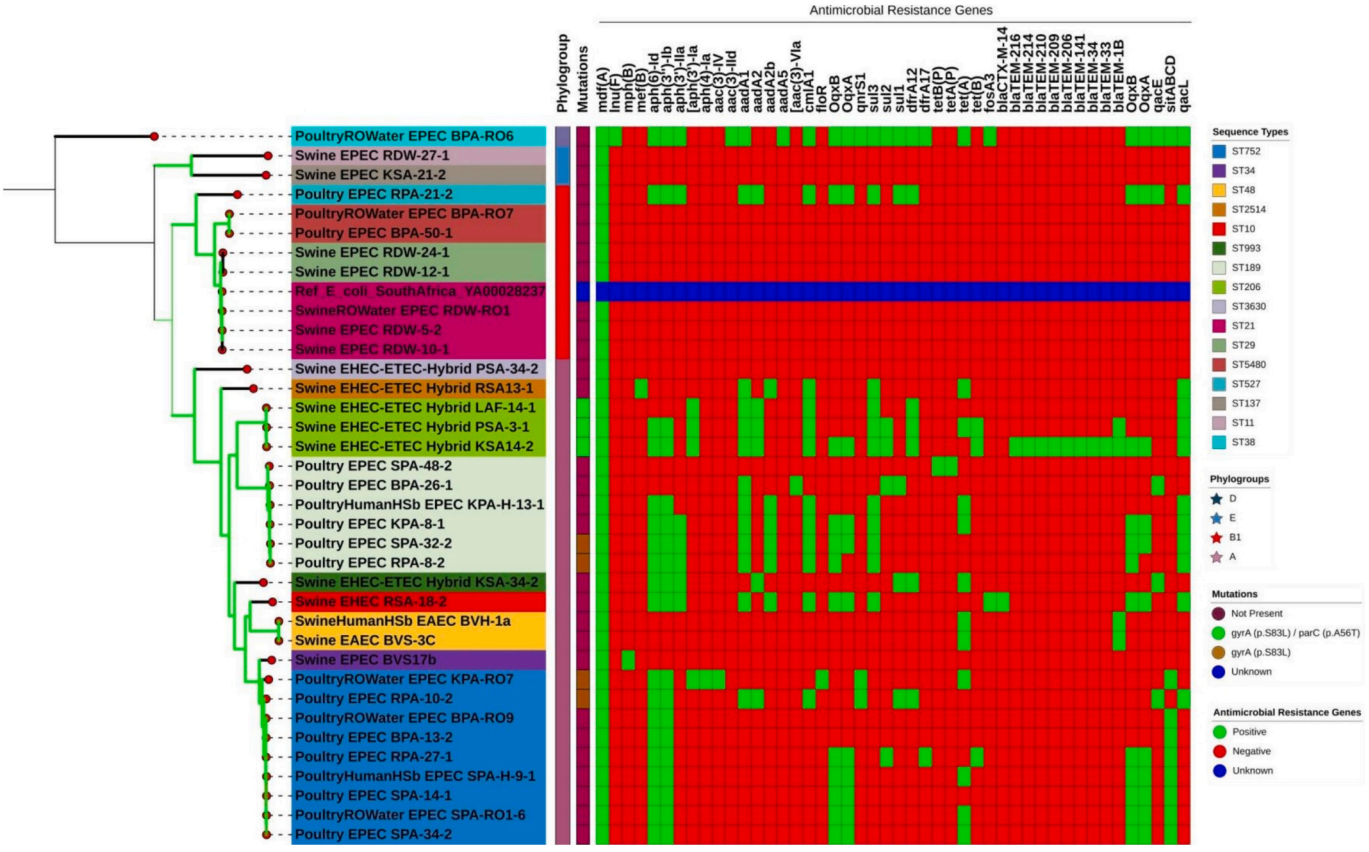

Fig. 5. Whole genome based phylogenetic relationships of PEC isolates and heatmaps of sequence types, phylogroups, mutations and antimicrobial resistance genes. The reference genome was *Escherichia coli* strain YA00028237. The tree was bootstrap and midpoint rooted.

Table 2
Profiles and genomic characteristics representative PEC isolates.

PEC isolates	Source	Human pathogen (score)	Sequence type	fimH type	Serogroup (WGS) O:H	Phylogroup
PSA-34-2	Swine	Yes (0.933)	3630	fimH27	O159:H16	A
RDW-RO1	Environmental	Yes (0.929)	21	fimH440	O26:H11	B1
RPA-8-2	Poultry	Yes (0.931)	189	fimH54	O136:H26	A
RPA-21-2	Poultry	Yes (0.929)	527	fimH1384	O115:H8	B1
RDW-10-1	Swine	Yes (0.929)	21	fimH440	O26:H11	B1
RDW-12-1	Swine	Yes (0.929)	29	fimH973	O26:H11	B1
SPA-48-2	Poultry	Yes (0.929)	189	fimH54	O80:H26	A
BPA-RO7	Poultry	Yes (0.937)	5480	fimH86	O103:H8	B1
SPA-34-2	Poultry	Yes (0.932)	752	fimH24	O123/O186:H40	A
SPA-32-2	Poultry	Yes (0.929)	189	fimH54	O136:H26	A
KPA-RO7	Environmental	Yes (0.93)	752	fimH24	UT:H31	A
KPA-8-1	Poultry	Yes (0.931)	189	fimH54	UT:H26	A
BPA-RO6	Environmental	Yes (0.913)	38	fimH65	O88:H18	D
KSA14-2	Swine	Yes (0.93)	206	fimH23	O141:UT	A
BVS-3C	Swine	Yes (0.928)	48	fimH400	O121:H11	A
BVS-17b	Swine	Yes (0.938)	34	fimH23	O1:H33	A
LAF-14-1	Swine	Yes (0.935)	206	fimH23	O141:UT	A
SPA-14-1	Poultry	Yes (0.933)	752	fimH24	O186/O123:H40	A
KPAH-13-1	Hand swab	Yes (0.931)	189	fimH54	UT:H26	A
RSA13-1	Swine	Yes (0.925)	2514	fimH54	O100:H20	A
BVH-1a	Hand swab	Yes (0.928)	48	fimH400	O121:H11	A
RDW-5-2	Swine	Yes (0.93)	21	fimH440	O26:H11	B1
KSA-21-2	Swine	Yes (0.921)	137	fimH331	O145:H28	E
SPA-RO1-6	Environmental	Yes (0.933)	752	fimH24	O186/O123:H40	A
BPA-50-1	Poultry	Yes (0.937)	5480	fimH86	O103:H8	B1
RDW-27-1	Swine	Yes (0.916)	11	fimH82	O157:H7	E
PSA-3-1	Swine	Yes (0.929)	206	fimH23	O141:UT	A
BPA-13-2	Poultry	Yes (0.934)	752	fimH24	O186/O123:H40	A
RPA-10-2	Poultry	Yes (0.923)	752	fimH24	O145:H40	A
BPA-26-1	Poultry	Yes (0.928)	189	fimH54	O80:H26	A
RSA-18-2	Swine	Yes (0.934)	10	fimH23	O50:H32	A
RPA-27-1	Poultry	Yes (0.927)	752	fimH24	O186/O123:H40	A
RDW-24-1	Swine	Yes (0.929)	29	fimH973	O26:H11	B1
KSA-34-2	Swine	Yes (0.933)	993	fimH604	O100:H30	A
SPAH-9-1	Hand swab	Yes (0.932)	752	fimH24	O186/O123:H40	A
BPA-RO9	Environmental	Yes (0.934)	752	fimH24	O186/O123:H40	A

UT: untypable.

one EPEC strain assigned to ST38 were detected in swine and environmental run-off water each (Table 2).

The phylogenomic analysis of the PEC isolates showed the diversity of circulating strains in swine, poultry, environmental run-off water and abattoir workers (Fig. 5). The majority of the PEC strains [69.4 % (25/36)] belonged to the commensal *E. coli* phylogroup A, while 22 % (8/36) were classed under phylogroup B1 according to ClermonTyper. The EPEC strains recovered from swine (KSA-21-2, RDW-27-1) and poultry facility run-off water (BPA-RO6) belonged to the virulent extra-intestinal phylogroup E and D, respectively (Table 2).

Table 3
Mobile genetic elements and associated virulence/antimicrobial resistance genes detected in the PEC isolates.

Plasmids	Transposons	Insertion sequence	Antimicrobial resistance genes [(n)]	Virulence factors [(n)]
IncFIC(FII)				*anr* [9], *traT* [4]
IncFII				*anr* [4], *toxB* [1]
IncFIB (AP001918)				*pap* [2], *hlyF* [8], *ompT* [8], *estC* [1], *traT* [1], *anr* [2], *espP* [1], *etp* [1], *ehxA* [1]
IncFII(29)			*aph(6)-Id* [1], *aph(3″)-Ib* [1], *sul2* [1]	*anr* [4], *traT* [3]
IncB/O/K/Z				*toxB* [3]
IncHI2/IncHI2A			*aph(6)-Id* [1], *aph(3″)*-Ib [1]	*terC* [1]
IncI1		IS*Sbo1*	*tet(A)* [3]	
	Tn*6082*	IS*102*	*aph(6)-Id* [5], *aph(3″)-Ib* [5], *tet(A)* [1]	*terC* [1]
	MITEEc1			*nleA* [3], *fdeC* [4], *terC* [13], *astA* [1], *hra* [1], *yeh* [2], *aamR* [1], *espB* [2], *espA* [2], *tir* [2], *eae-g01-gamma* [2]
		IS*903*	bla_{TEM-1B} [2]	
		ISEic2		*aamR* [5], *astA* [14], *eae-b01a-beta* [1], *tir* [1], *espA* [1], *espB* [1]
		IS*102*	*aph(6)-Id* [1], *aph(3″)-Ib* [1], *mph(B)* [1], *tet(B)* [1], *aph (3′)-Ia* [1]	
		IS*Kpn19*	*qnrS1* [2]	
		IS*6100*	*aac(3)*-Via [1], *qacE* [1], *sul1* [1], *aadA1* [1]	
		IS*26*	*aadA1* [1], *sul3* [1], *aadA2b* [1], *qacL* [1], *cmlA1* [1], *mph (B)* [1]	
		IS*Ec59*	*aph(4)-Ia* [1], *aac(3)-IV* [1]	
		IS*Kox3*	*sitABCD* [1]	*sitA* [1], *iucC* [1], *iutA* [1]
		IS*Vsa3*	*sul2* [1]	

Superscript: number of times WGS identified AMR/virulence factor gene.

3.8. Mobile genetic elements

A total of 21 different plasmid replicon types were detected in PEC strains, with the majority [91.7 % (33/36)] harbouring two or more plasmids (Supplementary Fig. 2). Major plasmids were found in the following proportions: (i) IncFIB(AP001918) 61.11 % (22/36), (ii) p0111 33.3 % (12/36), (iii) IncFII(pSE11) 30.6 % (11/36) and (iv) Col (MG828) 27.8 % (10/36). Plasmid replicon types associated with MDR strains in this study were the: (i) IncF variants IncFIC(FII) [25.0 % (9/36)] and IncFII(29) [22.2 % (8/36)], (ii) IncH variants IncHI2 [25 % (9/36)] and IncHI2A [22.2 % (8/36)], (iii) Incl1 13.9 % (5/36) and (iv) IncX1 11.1 % (4/36) (Supplementary Fig. 2). Major co-occurrence of plasmids with other MGEs such as ISs and Tns, associated with AMR and virulence factor genes are shown in Table 3. The transposon Tn*6082* concurrently harboured the *aph(6)-Id* and *aph(3″)-Ib* genes in five isolates and also harboured the *tet(A)* gene in one isolate. Likewise, findings showed the insertion sequences, IS*903* and IS*Kpn19*, harboured the $bla_{\text{TEM-1B}}$ and *qnrS1* genes, respectively (Table 3).

4. Discussion

Zoonotic transfer of resistant and virulent bacteria among animals, the environment and humans heightens public health risks and disease burdens (Aslam et al., 2021). Contamination of meat and meat products may facilitate outbreaks of zoonotic bacteria via the food chain, as seen in the 2017 to 2018 Listeriosis outbreak in South Africa (Thomas et al., 2020). Utilizing a One Health approach is crucial for understanding the intricate relationships and transmission of zoonotic pathogens between human, animal and the environment (Gebreyes et al., 2014). However, there are limited studies on the circulating PECs from commercial abattoirs and farms across South Africa. This study investigated the molecular epidemiology of PEC strains from humans, poultry, swine, and environmental water samples in Gauteng and Limpopo provinces from May 2019 to August 2020, using phenotypic and genotypic methods, including M-PCR assays and WGS. The findings revealed diverse PEC strains with significant AMR and virulence genes circulating in South Africa.

A total of 542 samples were collected for this study and 701 presumptive PEC colonies identified using the selective CHROMagar™ STEC (CHROMagar Microbiology, France). Previous studies conducted in South Africa have shown that this media does not permit the growth of only EHEC isolates but also other PECs such as EPEC (Kalule et al., 2018; Bolukaoto et al., 2019). This finding enabled the inclusion of detected EPEC isolates identified by the M-PCR assay in this study. A higher prevalence of EHEC and EPEC was found in Gauteng province compared to Limpopo province, influenced by the fact that most sampled sites were in Gauteng. The M-PCR assay detected only one EHEC O157 serogroup in swine, harbouring the *stx2*, *eae*, and *hlyA* genes. This EHEC O157 serogroup is responsible for outbreaks of HC and HUS in humans worldwide (Sperandio and Nguyen, 2012). The low prevalence of EHEC O157 in this study concurs with other findings reported in South Africa (Abong'o and Momba, 2009; Bolukaoto et al., 2019). The M-PCR assay identified O26 as the most frequently detected EPEC serogroup in this study. Serogroup O26 is the second most significant EPEC serogroup associated with persistent diarrhoea in children in South Africa and worldwide (Gonzalez-Escalona et al., 2016; Smith et al., 2019).

The PFGE analysis revealed that PEC isolates from different sampling sites did not cluster in the same pulsotype, indicating homogeneity within each location. Three PEC isolates from poultry, human hand swabs and run-off water at the same site formed a minor pulsotype F. These findings highlight the risk of abattoir workers being exposed to PEC from animal sources, potentially facilitating community transmission through person-to-person contact or contaminated environmental water. Similar studies in Egypt and Ethiopia have found *E. coli* and *Salmonella* spp. on hand swabs of abattoir workers (Abd-Elaleem et al., 2014; Geresu and Desta, 2021).

Utilizing WGS data, PEC isolates were classified into EHEC and EPEC, aligning with M-PCR assay results. However, two isolates initially negative for virulence factors but positive for PEC serogroup by M-PCR were reclassified as EAEC. This discrepancy is due to the M-PCR assay not targeting EAEC-specific virulence factors. Moreover, WGS offers broader genomic coverage, making it a superior tool for detecting the PEC virulome (Malberg Tetzschner et al., 2020). The M-PCR assay failed to detect the *stx1* gene in EHEC isolates, except in the positive control (O157, *stx1*, *stx2*, *eae*, *hlyA*). However, a discrepancy emerged upon analyzing WGS data: the VFDB tool identified both *stx1* and *stx2* genes in 71.4 % of EHEC isolates, whereas the CGE VirulenceFinder tool only detected the *stx2* variants. To improve the credibility and reliability of sequencing results, it is crucial to harmonize genomic databases for consistent and accurate detection.

The study identified three major subtypes of *stx2* namely: (i) *stx2b*, (ii) *stx2e* and (iii) the novel *stx2k* from swine sources. The EHEC isolates harbouring the *stx2* gene are more virulent, causing HC and HUS, compared to those with *stx1* (Llarena et al., 2021). Foodborne outbreaks in South Africa have been associated with EHEC isolates carrying the *stx2* gene variant, which has been linked with HUS in children aged between 8 months and 5 years (Smith et al., 2019). This is the first documentation of the EHEC *stx2k* subtype of Shiga toxin in South Africa and potentially in Africa. The *stx2k* subtype was first reported in China in diarrheal patients, animals (including swine) and raw meat (Hughes et al., 2019). The *stx2k* positive isolate in this study was an EHEC/ETEC hybrid pathotype as observed in the study by Hughes et al. (2019), which suggests the rise of new virulent strains. Further research is needed to understand the role of *stx2k* in HC and HUS. Additionally, an EHEC O157 isolate that harboured the complete EHEC plasmid *toxB*, *katP*, *etp*, *ehxA* and *espP* genes showed *stx2* presence with M-PCR but not with WGS, likely due to loss of *stx*-encoding bacteriophages during culturing, or other factors which affect *stx2* bacteriophage stability such as improper preservation and unstable storage temperature (Rode et al., 2011; Madoroba et al., 2022). A study by Castro et al. (2021) found that 22 *E. coli* isolates tested positive for the *stx* gene with PCR but tested negative with WGS due to harbouring truncated *stx* fragments. This may explain the failure to detect the *stx2* gene in the EHEC O157 isolate in the current study. Consequently, this isolate was reclassified as EPEC O157.

In this study, two EAEC isolates harboured the putative virulence gene *aaiC*, a chromosomal type VI secretion system gene. The EAEC is commonly associated with intestinal inflammation, growth impairment in children and traveller's diarrhoea in adults (Bamidele et al., 2019; Das et al., 2021). Although EAEC typically expresses the transcriptional activator of aggregative adherence fimbria I *AggR* gene, which regulates several virulence genes including *aaiC* (Rogawski et al., 2017), neither of the two EAEC isolates in this study had the *AggR* gene. However, EAEC can acquire other virulence genes that enhance its pathogenicity, as demonstrated by the 2011 gastroenteritis outbreak in Germany where EAEC acquired EHEC *stx2* bacteriophages (Bielaszewska et al., 2011).

Toxin genes such as *pic*, *hlyF* and *cvaC*, as well as fitness and iron uptake genes like *estC*, *sitA*, *iucC*, *iutA*, *tsh* and *iroN*, were significantly associated with *eae-ε* variants of EPEC isolates from poultry, abattoir worker hand swabs and run-off water. These genes are linked to the Colicin V virulence plasmid (ColV), which encodes factors crucial for iron uptake, serum survival and resistance to phagocytosis (Hammad et al., 2022). The ColV virulence factors bolster host invasion and colonisation while evading host defenses, potentially causing severe diseases such as septicaemia and neonatal meningitis (Hammad et al., 2022). Thus, the presence of ColV in these EPEC isolates increases their pathogenic potential and capacity to cause human infections.

An interesting finding was the presence of the AraC negative regulator, *anr* gene, in 61.1 % of PEC isolates across all three pathotypes. This gene negatively controls over 500 genes in PECs, including virulence and adherence genes like the T3SS genes in EPEC isolates, significantly

diminishing adhesion and the generation of A/E lesions in human intestinal tissues (Rodriguez-Valverde et al., 2023). This may explain the infrequent reports of foodborne PEC outbreaks in South Africa, despite the high prevalence of PEC in livestock. The *anr* gene might reduce the pathogenicity of PEC isolates in South Africa, warranting further investigation.

In this study, an array of AMR genes was detected using both phenotypic and genotypic tests. Six PEC isolates resistant to cephalothin, a first-generation cephalosporin was reported. One EHEC isolate from swine exhibited resistance to ten antimicrobials, including second and third-generation cephalosporins like ampicillin, aztreonam, cefotaxime, ceftazidime, and cefuroxime. An EPEC isolate from environmental run-off water showed resistance to eight antimicrobials, including ciprofloxacin, norfloxacin, and tobramycin. These findings are consistent with previous reports from South Africa and China, which also found high resistance to antimicrobials like cefuroxime, ampicillin, and ceftazidime in EHEC and EPEC isolates from clinical, environmental and poultry sources (Bolukaoto et al., 2021; Shafiq et al., 2021). One EHEC isolate from Gauteng province exhibited phenotypic resistance to cephalosporins and harboured the CTX-M gp 9 and $bla_{\text{CTX-M-14}}$ AMR genes, as confirmed by both M-PCR assay and WGS. The $bla_{\text{CTX-M-14}}$ gene has previously been reported in clinical isolates from Gauteng province and in swine isolates from abattoirs in KwaZulu-Natal province, South Africa (Mbelle et al., 2019; Founou et al., 2022). Interestingly, the EHEC isolate in this study exhibited similar AMR characteristics to those found in PEC isolates observed by Founou et al. (2022) in KwaZulu-Natal province, indicating that MDR EHEC isolates with comparable traits were circulating within these two South African provinces around the same time.

Three MDR EHEC isolates from swine samples exhibited QRDR mutations in the *gyrA*(p.S83L) and the *parC*(p.A56T) genes conferring high-level resistance to fluoroquinolones and carried the SMR efflux pump *qacL* gene, which confers resistance to antiseptics and disinfectants, including QACs. The presence of the *qacL* gene in bacteria pathogens is linked to increased selective pressure and the development of MDR (Gregorchuk et al., 2020). According to Buffet-Bataillon et al. (2016), QAC exposure can prompt efflux pump overexpression and QRDR mutations, which might support the findings in this study. The high prevalence (66.7 %) of MDR PEC isolates in this study may result from selective pressure and adaptation over time. Recent research indicates that the evolution of AMR genes and bacterial fitness, rather than antimicrobial consumption alone, is the primary driver of AMR in agricultural settings (Ogunlana et al., 2023).

All the MDR STs grouped under the commensal phylogroup A except the BPA-RO6 ST38 strain recovered from a run-off water sample, which belongs to the virulent phylogroup D. These commensal PEC isolates can become pathogenic to animals and humans due to the array of virulence factors and AMR genes they harbour and can be transfer to other pathogenic and non-pathogenic strains in the environment (Bengtsson-Palme et al., 2018). Reports from various countries have linked ST752 and ST189 strains belonging to the newly reported EPEC non-classical O123/186 and O80 serogroups with the spread of resistance to third-generation cephalosporin, sulphonamide, tetracycline, trimethoprim and quinolones (Cointe et al., 2018; Mo et al., 2020). However, none of the strains in the current study, belonging to ST752 and ST189 carried a third-generation cephalosporin-resistant gene, although a significant association with resistance to sulphonamide, tetracycline, phenicol and quinolones was observed.

The phylogenetic analysis showed that EHEC ST206 strains (LAF-14-1, PSA-3-1 and KSA14-2) recovered from a swine farm and two different swine abattoir sites in Gauteng province were closely related and belonged to serogroup O141 (Fig. 5). This finding further establishes the epidemiological transmission route of PEC along the farm-to-fork chain. Infection of farm animals primarily takes place in the farm environment, while faulty processing at the abattoir plants might lead to food contamination and subsequent colonisation of the human host by PEC (Abdalla et al., 2021).

A repertoire of MGEs found in PEC isolates harbouring AMR and virulence genes can play a crucial role in the transfer of virulence traits from pathogenic to non-pathogenic intestinal pathogens on ingestion of contaminated products (Singh et al., 2018). The $bla_{\text{CTX-M-14}}$ positive EHEC isolate in this study harboured the IncFIC(FII) and IncFIB plasmid variants. In China, the plasmid replicon IncF and its variants were associated with the spread of $bla_{\text{CTX-M}}$ in paediatric patients (Patil et al., 2019). The IncF plasmid replicon types and its variants have been recognised as the global disseminators of AMR genes in humans, animals and the environment (Founou et al., 2022; Pitout and Chen, 2023). One PEC isolates encoded the transposon Tn*6082* that harboured the *tet(A)* gene. Studies have reported Tn*6082* harbouring the streptomycin *aph(6)-Id* and *aph(3″)-Ib* resistance operon, in *Klebsiella pneumoniae* and *Salmonella enterica* (Sampei et al., 2010; Wang et al., 2015) and recently in *Pseudomonas aeruginosa* (Mehrotra et al., 2023). However, there is currently limited knowledge regarding Tn*6082* harbouring the *tet(A)* gene. Another interesting finding of this study was the identification of the ESBL ampicillin $bla_{\text{TEM-1B}}$ gene which was harboured by the IS*903* insertion sequence. Other reports around the world have associated IS*903* predominantly with the spread of $bla_{\text{CTX-M}}$ in clinical, environmental and animal isolates (Said et al., 2016; Tayh et al., 2016; Tadesse et al., 2018). These findings showed that ISs and Tns elements are evolving with increasing capacity to spread AMR genes among bacterial pathogens.

It is noteworthy that this study represents the first documentation of the EHEC *stx2k* subtype in swine from Africa and highlighted the presence of diverse serogroups, virulence genes, STs and AMR genes in PECs. This underscores the potential threat of zoonotic pathogens to human health. This study also emphasizes the ongoing risk posed by the evolution of MGEs and the increased stability of emerging virulence and AMR genes in PECs. However, certain limitations were reported such as the discrepancy in the detection of *stx1* gene by virulence databases used and the inability of WGS to recover the *stx2* gene in one PEC isolate. Additionally, only the seven isolates positive for ESBL with the DDST were further analyzed for AST using the automated WalkAway 40 plus MicroScan system (Beckman Coulter Inc., California, USA), leading to an incomplete AST profile in PEC isolates. Given that PEC isolates may harbour AMR genes without expressing them, future studies should prioritize obtaining complete AST profiles for PEC isolates analyzed with WGS.

5. Conclusion

In conclusion, the findings in this study emphasise the importance of continuous monitoring of foodborne pathogens because they have the potential to evolve into virulent strains capable of causing large outbreaks. Furthermore, the shortage of veterinarians and para-veterinarians in South Africa to institute oversight of the use of antimicrobials among farmers, especially in rural areas where access to veterinary care and services is limited, is a major concern. There is a need for the enforcement of custom-built management and hygienic practices in food processing facilities by governing agencies responsible for monitoring food safety and public health. Interventions, using the whole-of-society approach (government, policy makers, private sector, value chain stakeholders, etc.) to control the spread of AMR pathogens are needed, therefore emphasising the need for a One Health approach to research.

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CRediT authorship contribution statement

Samuel T. Ogundare: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Folorunso O. Fasina:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **John-Paul Makumbi:** Writing – review & editing, Investigation. **Gerbrand A. van der Zel:** Visualization, Resources, Project administration. **Peter F. Geertsma:** Resources, Project administration. **Marleen M. Kock:** Writing – review & editing. **Anthony M. Smith:** Writing – review & editing, Resources, Investigation, Data curation. **Marthie M. Ehlers:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Samuel T. Ogundare reports financial support was provided by National Research Foundation. Anthony M Smith reports financial support was provided by United Kingdom Department of Health and Social Care. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in this study are included in the article and supplementary material. Additional information can be requested from the corresponding author.

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Appendix A. Supplementary data

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