

# CHARACTERIZATION AND EPIDEMIOLOGICAL SUBTYPING OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* ISOLATED FROM THE BEEF PRODUCTION CHAIN IN GAUTENG, SOUTH AFRICA

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## Highlights

- Gene combinations *stx*<sub>1</sub>/*stx*<sub>2</sub> + *eaeA* was detected at every stage of the beef chain.
- The 35% MDR could be an indicator of levels of antimicrobial use in livestock.
- PFGE revealed a high genomic diversity of non-O157 STEC strains.
- Evidence of horizontal transmission and STEC strain recirculation in the beef chain.

## **ABSTRACT**

In South Africa, there is a shortage of epidemiologic data on Shiga toxin-producing *Escherichia coli* (STEC) in the beef production chain. This study was conducted to characterize STEC isolates originating from three studies conducted in a cattle feedlot, beef abattoirs and retail outlets in Gauteng province, South Africa. Polymerase chain reaction was used to detect virulence genes, the Epsilonometer test to assess antimicrobial susceptibility, pulsed-field gel electrophoresis (PFGE) to investigate genetic relatedness of isolates, and conventional serotyping for phenotypic identification. Amongst the 86 STEC isolates, the *eaeA* gene was detected in 20 (23%), and 26 different serogroups were identified, including the clinically important O8, O174, O2, O20 and O117. The majority of the isolates (95%; 82/86) exhibited resistance to one or more antimicrobial agents, and 30 of the isolates (35%) exhibited multi-drug resistance (MDR), being resistant to at least three antimicrobial classes. The PFGE patterns showed a highly diverse but related STEC population, with 45 distinct patterns and evidence of horizontal transmission along the beef production chain. This is significant because it demonstrates continual environmental contamination and risk of contamination along the beef production chain and the food chain. To our knowledge, this is the first study that provides evidence of horizontal transmission of STEC along the beef production chain in South Africa. This epidemiological information could facilitate the development of a proactive strategy for reducing potential foodborne outbreaks and transmission of antimicrobial resistant pathogens in the food chain.

**Key words:** Antimicrobial resistance in STEC, Non-O157 STEC, Pulsed-field gel electrophoresis, Epidemiological subtyping of STEC, Beef production chain

**Running title:** Subtyping of STEC in the beef chain

## 1. Introduction

*Escherichia coli* comprises part of the normal flora in human and animal intestinal tracts which are beneficial to the host. However, some strains become pathogenic through lateral acquisition of genetic materials such as Shiga toxin genes (*stx*<sub>1</sub> and *stx*<sub>2</sub>) encoded on prophages into the *E. coli* chromosome, thereby possessing the capacity to cause disease and public health burdens worldwide (Lacher et al., 2018; FAO/WHO 2019). Clinical illness ranges from mild diarrhoea to severe haemorrhagic colitis (bloody diarrhoea) and may progress to thrombotic thrombocytopenic purpura (TTP) and haemolytic-uremic syndrome (HUS) (Lacher et al., 2018; FAO/WHO 2019). Asymptomatic cattle are the main reservoir of the pathogen and can shed high numbers that result in contamination of the farm environment (Buncic et al., 2014). This could lead to a sequence of STEC contamination at every stage of the beef production chain, from on-farm production activities, to abattoir slaughter processes, to the post-harvest stage (manufacturing and preparation of beef and beef products) in commercial establishments, including retail outlets (Buncic et al., 2014; Nastasijević et al., 2014).

Livestock production in South African is an evolving sector of agriculture, boosted by available agricultural land, and contributes about 40% to agricultural income (DAFF, 2018). The beef industry is ranked as the second fastest growing industry in the agricultural sector after the broiler industry (DAFF, 2019). In Gauteng province, cattle production is considered as an emerging industry when compared with provinces like Eastern Cape, KwaZulu-Natal and Free State where cattle production is a major commercial activity (DAFF, 2019). A dual production system exists, including the high-level commercial system and the small-scale production system (DAFF, 2017). Primary production includes animal pasture grazing and secondary production which involves feedlot finishing of animals (DAFF, 2018).

The faecal contamination of the environment generated from production systems also serves as a potential source for the dissemination of antimicrobial resistant (AMR) bacteria (Landers et al., 2012). Bacteria can develop resistance to antimicrobial agents either through the natural process of mutation or the acquisition of resistance genes from other bacteria (Wintersdorff et al., 2016). Transmission of AMR bacteria from animals to humans is influenced by a complex mix of factors and the beef production chain has been implicated as an important route of transmission of AMR bacteria to humans (Van den Honert et al., 2018).

South Africa ranks among rising economies like China, Brazil, India and Russia, which are considered hotspots for antimicrobial resistance due to exponential growth in intensive livestock production and a consequent upsurge in antimicrobial use (Grace et al., 2015; Van Boeckel et al., 2015). In livestock production, antimicrobials are used to treat and prevent disease, and as growth promoters, and over the years their misuse, particularly in intensive production systems, has contributed to the emergence of novel AMR bacterial strains due to selective pressure on the normal flora (Capita et al., 2013; Moyane et al., 2014). The abundance of *E. coli* in the animal intestinal tract constitutes an enormous pool of resistance genes (Aminov and Mackie, 2007; Capita et al., 2013); this, coupled with *E. coli* genome dynamics and plasticity, promotes the integration of virulence and/or resistance genes which have the capacity to generate hybrid strains (Adefisoye and Okoh, 2016; Lacher *et al.*, 2018).

The availability of epidemiologic data providing evidence of horizontal transmission along the different stages of the beef production chain is one of the key prerequisites to risk assessment and the development of mitigation strategies for STEC-contaminated beef and beef products from reaching the table of consumers (Nastasijević et al., 2014). Molecular subtyping techniques such as pulsed field gel electrophoresis (PFGE) provide valuable confirmatory information for epidemiological investigations and PFGE has been used to track foodborne bacterial pathogens along the food production chain (Peters, 2009; Lytsy et al., 2017).

In South Africa, there are major gaps in the available data that link all the important stages in the beef production chain which could facilitate the development of a proactive strategy for reducing potential foodborne outbreaks. This study aimed to provide relevant data on STEC through the characterization and epidemiological subtyping of STEC isolates recovered at every stage along the beef production chain. The specific objectives were (1) to determine the prevalence and pattern of resistance to commonly used antimicrobial agents in humans and animals in South Africa, (2) to determine the serogroups of STEC strains along the beef production chain using phenotypic subtyping techniques, and (3) to epidemiologically characterize STEC strains recovered at different stages along the beef production chain using PFGE as a molecular subtyping technique.

## **2. Materials and methods**

### **2.1. Sources of isolates**

Three independent studies were conducted during November 2015 to February 2017 to determine the prevalence of STEC and associated risk factors in Gauteng, South Africa. These included two cross-sectional studies involving 12 beef abattoirs (5 low- and 7 high-throughput) (Onyeka et al., 2021) and 31 retail outlets (17 large chain supermarkets and 14 butchereries) (Onyeka et al., 2020), and one longitudinal investigation conducted at a cattle feedlot (Onyeka 2019). Samples collected included faeces by rectal grab, hide and carcass swabs, and swabs of walls and floors of slaughter halls of beef abattoirs. Retail outlet samples consisted of raw beef products comprising brisket, mincemeat, and boerewors (spiced sausage), and ready-to-eat (RTE) beef products comprising biltong (dried, salted beef) and cold meat. Only field broth cultures that were PCR-positive for *stx*<sub>1</sub> or *stx*<sub>2</sub> or both were considered positive for STEC and were cultured to isolate STEC strains. For the feedlot

study, only 30 STEC positive field samples yielded only 38 isolates and only 15 were serotypeable. For the abattoir study, of the 147 *stx*-positive field broth samples, only 33 STEC isolates were recovered, and 14 isolates were typeable. For the red meat retail study, of the 76 *stx*-positive field broth cultures, only 38 isolates were recovered and 26 were typeable. Overall, of the 253 STEC positive field broth cultures, only 109 isolates were recovered, because subculture of other samples was negative. Also, only 55 isolates were typeable and 54 were O-serogroup non-typeable (ONT). For the current study, only 86 pure isolates were recovered and selected for the AMR study, of which 34 originated from the feedlot, 28 from abattoirs and 24 from retail outlets.

## **2.2. Detection of virulence genes**

DNA templates were prepared as crude lysate. Briefly, a loopful of pure culture of each isolate was suspended in 1 ml molecular grade water, vortexed until thoroughly mixed and centrifuged (Merck, SA) for 10 min at 10,000 x g. The supernatant was decanted and 200 ml Tris-EDTA buffer was added and vortexed until properly mixed. The suspension was boiled for 15 min at 95°C on a heating block (Merck, New Jersey, USA) and thereafter allowed to cool at room temperature and the supernatant stored at -20°C until required. Multiplex polymerase chain reaction (mPCR) was conducted to detect the presence of *stx*<sub>1</sub>, *stx*<sub>2</sub>, *eaeA* and *hlyA* genes as previously described (Onyeka et al., 2020).

## **2.3. Conventional serotyping of isolates**

Isolates were serotyped at the Centre for Enteric Diseases, National Institute for Communicable Diseases (CED-NICD), Johannesburg, South Africa, using the conventional serotyping method (Ørskov and Ørskov, 1984). Only the O-surface antigen (lipopolysaccharides) serotyping was conducted using the complete range of *E. coli* O POOL of polyvalent antisera: AA-WW from Statens Serum Institute (SSI) (Copenhagen, Denmark). The method used followed the

manufacturer's instructions according to methods described by Ørskov et al., (1984). Saline was used as a negative control and 2014-2015 VTEC EQA-*E. coli* RR18-3022 O157 (*eae*, *stx1a*, *stx2a*) as positive control. Isolates were reported as either typable or O-non-typeable (ONT).

#### **2.4. Antimicrobial resistance testing**

The selection of antimicrobial agents used in this study was based on two criteria, the commonly used antimicrobial agents in food animals in South Africa (Eagar et al., 2012; Moyane et al., 2013), and the clinically relevant antimicrobial agents used in humans in South Africa (Rossiter, 2016). Epsilometer test (Etest) strips (bioMérieux, Marcy l'Etoile, France) of 17 antimicrobial agents were used to test a total of 86 isolates. The antimicrobial agents and their respective minimum inhibitory concentration (MIC) ranges and breakpoints are listed in Table 1.

The protocol used was as described in the Etest package instructions ([http://www.illexmedical.com/files/E-test-Package-Insert/AST\\_WW.pdf](http://www.illexmedical.com/files/E-test-Package-Insert/AST_WW.pdf)) and CED-NICD laboratory protocol. Briefly, three to five (3-5) pure colonies were suspended in sterile saline (0.85% NaCl) to obtain a turbidity equivalent to 0.5 McFarland Standard (Remel, USA). Suspension was uniformly swabbed on a Mueller–Hinton agar (MHA) plate using a plate rotator. Two Etest strips were placed onto the MHA plates, and aerobically incubated at 37°C for 18-24 h.

Reference test quality control (QC) was performed using *Escherichia coli* ATCC 25922 and ATCC 29213 *Staphylococcus*. The Clinical and Laboratory Standard Institute (CLSI) guidelines (CLSI, 2015a; 2015b) for MIC breakpoints were used for interpretation, as per the manufacturer's instructions. Where the breakpoint of the antimicrobial agents was not observed, then the CLSI guideline for human isolates was used. For this study, isolates that produced intermediate values were considered resistant.

**Table 1: Description of 18 antimicrobial Epsilon test strips used to test 86 Shiga toxin-producing *Escherichia coli* isolates recovered from the beef production chain in Gauteng, South Africa**

Antimicrobial Class	Antimicrobial agent tested	ID	Etest strip dilution gradient (µg/ml)	Resistance breakpoint
Penicillins	Ampicillin	AM	0.016-256	32
	Amoxicillin-clavulanic acid	XL	0.016-256	32
Cephems	Ceftazidime	TZ	0.016-256	4
	Ceftriaxone	TX	0.016-256	4
	Cefepime	PM	0.016-256	16
Carbapenems	Imipenem	IP	0.02 - 32	4
Aminoglycosides	Kanamycin	KM	0.016-256	64
	Streptomycin	SM	0.064-1024	64
Quinolones	Nalidixic acid	NA	0.016-256	32
	Ciprofloxacin	CI	0.02 - 32	1
Folate pathway inhibitors	Trimethoprim/ Sulphamethoxazole (Cotrimoxazole)	TS	0.02 - 32	4
	Trimethoprim	TR	0.02 - 32	16
	Sulphamethoxazole	SX	0.064 - 1024	5
Macrolides	Azithromycin	AZ	0.016-256	32
Nitrofurans	Nitrofurantoin	NI	0.032-512	128
Phenicol	Chloramphenicol	CL	0.016-256	32
Tetracyclines	Tetracycline	TC	0.016-256	16

## 2.5. Pulse field gel electrophoresis (PFGE)

PFGE patterns were used to compare the genetic relatedness of isolates belonging to the same serogroup. A standard protocol for non-O157 STEC from PulseNet (PulseNet, CDC, 2017) and SOP (document NIC0301) of CED were used. Briefly, 55 serotypeable STEC isolates were suspended in cell suspension buffer, proteinase K (Roche diagnostics, Germany) was added and plugs were prepared. Plug slices were digested with restriction enzyme *Xba*I (Roche diagnostics, Germany). PFGE was performed in a CHEF-DRII electrophoresis system (Bio-



Rad); in each run the *Salmonella enterica* Braenderup (strain H9812) standards were included. The gels were stained with ethidium bromide staining solution (Sigma) and banding patterns were captured using the Bio-Rad gel documentation system (Bio-Rad, Hercules, CA, USA). TIFF images of the band patterns were analysed with BioNumerics, version 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Between lanes and between gel band normalization was achieved using *Salmonella enterica* serotype Braenderup H9812 as the size calling reference marker. Band profile analysis was performed using the dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA) to construct dendrograms, set at 5% band tolerance and 1.5% optimization.

## **2.6. Statistical analysis of AMR data**

The prevalence of antimicrobial resistance (AMR) in the 86 STEC isolates was analysed by sample source. Prevalence of AMR with exact binomial 95% confidence interval was estimated for each antimicrobial agent. The association between virulence genes and occurrence of AMR was assessed using Fisher's exact tests. All analyses were performed using Stata14 (StataCorp, College Station, TX, U.S.A.), and the significance level was set to 5%.

# **3. RESULTS**

## **3.1. Virulence gene profiles and combinations**

Of the 86 isolates, only 55 (50%) were typable, representing 26 non-O157 serogroups including the following clinically relevant serogroups: O2 (two isolates), O8 (five isolates), O117 (two isolates), O174 (one isolate) and O178 (three isolates). The clinically important serogroups were highest in red meat retail (6 isolates), followed by abattoir (5 isolates) and feedlot (2 isolates). Overall, the gene *stx*<sub>1</sub> had the highest frequency (24%; 21 isolates), followed by *stx*<sub>1</sub>+*stx*<sub>2</sub> combination (19%; 16 isolates), *stx*<sub>2</sub> (17%; 15 isolates) and 8% carried

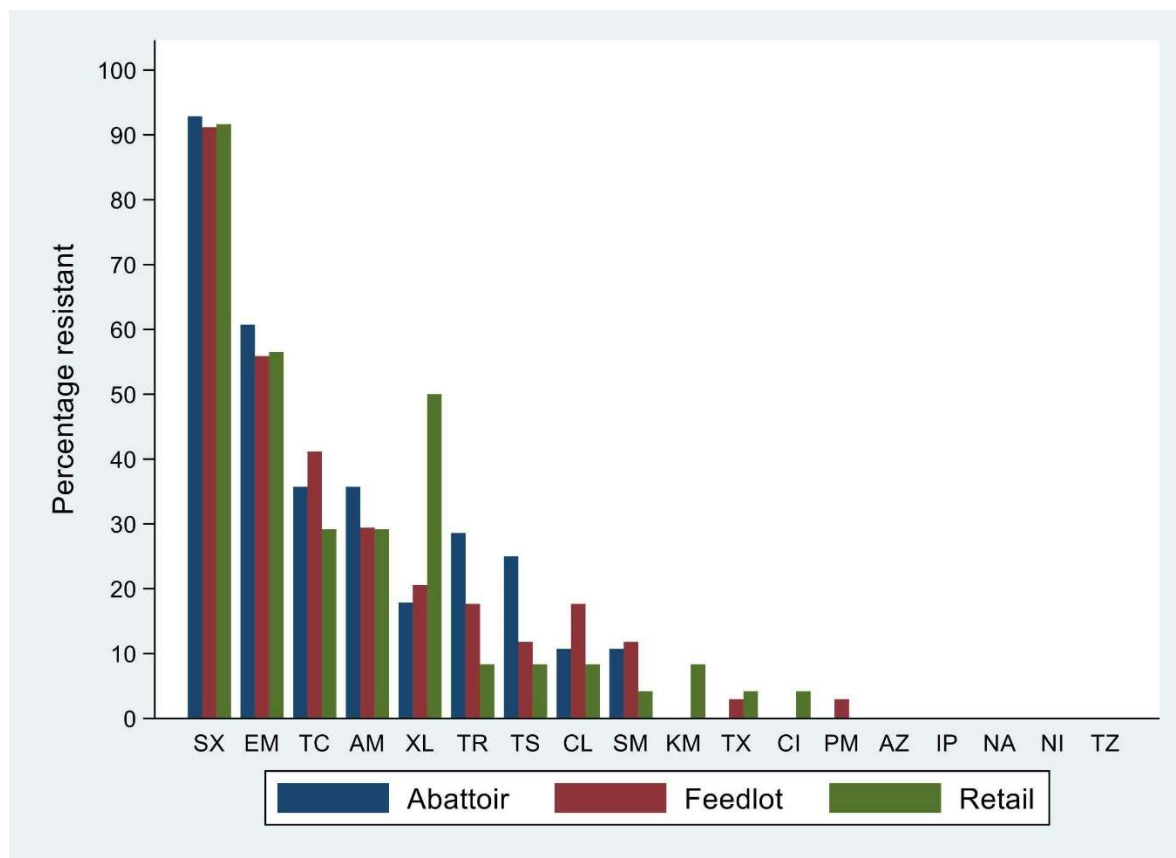
**Table 2: Serogroups, virulence gene profiles and combinations of 86 Shiga toxin-producing *Escherichia coli* isolates recovered from the beef production chain in Gauteng, South Africa**

Serogroups	Virulence factor profile	No. of STEC isolates		
		Feedlot	Abattoir	Retail
O2	<i>stx<sub>1</sub>, stx<sub>2</sub></i>	0	1	1
O4	<i>stx<sub>1</sub> + eaeA</i>	0	1	0
O6	<i>stx<sub>1</sub></i>	0	1	0
O8	<i>stx<sub>1</sub> + stx<sub>2</sub>, stx<sub>2</sub> + hlyA</i>	0	2	1
O10	<i>stx<sub>1</sub></i>	1	1	0
O13	<i>stx<sub>1</sub></i>	0	0	2
O16	<i>stx<sub>1</sub> + stx<sub>2</sub> + hlyA</i>	0	1	0
O20	<i>stx<sub>2</sub></i>	0	0	2
O24	<i>stx<sub>2</sub> + hlyA</i>	0	0	1
O36	<i>stx<sub>1</sub></i>	1	0	0
O39	<i>stx<sub>1</sub></i>	0	0	1
O41	<i>stx<sub>1</sub> + stx<sub>2</sub></i>	0	0	1
O68	<i>stx<sub>1</sub> + stx<sub>2</sub> + eaeA + hlyA</i>	1	0	0
O76	<i>stx<sub>1</sub></i>	0	0	1
O88	<i>stx<sub>1</sub> + stx<sub>2</sub></i>	1	0	0
O101	<i>stx<sub>1</sub> + eaeA</i>	1	0	0
O102	<i>stx<sub>2</sub></i>	0	1	0
O108	<i>stx<sub>1</sub> + stx<sub>2</sub> + hlyA, stx<sub>2</sub> + eaeA + hlyA</i>	2	0	0
O117	<i>stx<sub>1</sub> + stx<sub>2</sub> + hlyA, stx<sub>1</sub> + stx<sub>2</sub></i>	0	2	0
O132	<i>stx<sub>1</sub></i>	1	0	0
O140	<i>stx<sub>1</sub></i>	1	0	0
O150	<i>stx<sub>1</sub> + stx<sub>2</sub></i>	0	0	1
O168	<i>stx<sub>2</sub>, stx<sub>1</sub> + eaeA, stx<sub>1</sub> + stx<sub>2</sub></i>	3	1	0
O174	<i>stx<sub>2</sub> + eaeA</i>	0	0	1
O178	<i>stx<sub>2</sub></i>	2	0	1
O182	<i>stx<sub>1</sub> + stx<sub>2</sub></i>	1	0	0
Undetermined	<i>stx<sub>1</sub></i>	1	9	0
Undetermined	<i>stx<sub>1</sub> + eaeA + hlyA</i>	8	1	0
Undetermined	<i>stx<sub>1</sub> + hlyA</i>	1	0	0
Undetermined	<i>stx<sub>1</sub> + stx<sub>2</sub></i>	2	2	5
Undetermined	<i>stx<sub>1</sub> + stx<sub>2</sub> + hlyA</i>	3	3	0
Undetermined	<i>stx<sub>1</sub> + stx<sub>2</sub> + eaeA</i>	0	0	1
Undetermined	<i>stx<sub>2</sub></i>	2	1	4
Undetermined	<i>stx<sub>2</sub> + eaeA</i>	0	1	0
Undetermined	<i>stx<sub>2</sub> + eaeA + hlyA</i>	2	0	0
Undetermined	<i>stx<sub>2</sub> + hlyA</i>	0	0	1

the *stx<sub>2</sub>+eaeA* gene combination. Largely, the *eaeA* gene was detected in six different combinations found in 20 isolates (23%): *stx<sub>1</sub> + eaeA* (one isolate), *stx<sub>2</sub>+eaeA* (two isolates), *stx<sub>1</sub>+stx<sub>2</sub>+eaeA* (one isolate), *stx<sub>1</sub>+eaeA+hlyA* (12 isolates), *stx<sub>2</sub>+eaeA+hlyA* (three isolates), *stx<sub>1</sub>+stx<sub>2</sub>+eaeA+hlyA* (one isolate) (Table 2). There was no association between the virulence genes (*stx<sub>1</sub>*, *stx<sub>2</sub>*, *eaeA* and *hlyA*) and antimicrobial resistance.

### **3.2. Prevalence of AMR and multidrug resistance (MDR)**

In our study, AMR was defined as resistance to at least one antimicrobial agent, and MDR defined as “acquired non-susceptibility to at least one agent in three or more antimicrobial categories” (Magiorakos et al., 2012). The majority of the STEC isolates (82/86; 95%) exhibited resistance to at least one antimicrobial agent, and 92% (79/86; 95%CI: 84-97%) of the STEC isolates exhibited resistance to sulfamethoxazole, followed by 36% for tetracycline (31/86; 95%CI: 26-47%) (Figure 1). Thirty of the isolates (35%) exhibited MDR; one of the MDR isolates exhibited resistance to nine antimicrobial agents, four showed resistance to seven antimicrobial agents and one was resistant to six antimicrobial agents. All 86 isolates (100%) were susceptible to five antimicrobial agents (nalidixic acid, ceftazidime, nitrofurantoin, imipenem and azithromycin). Furthermore, we observed 99% susceptibility to ciprofloxacin and cefepime, and 98% susceptibility to kanamycin and ceftriaxone. AMR and MDR were observed in isolates across the beef production chain, i.e. feedlot, abattoir and red meat retail outlet. The multidrug resistance patterns for STEC isolates from the three sources is shown in Table 3.



**Figure 1: Phenotypic antimicrobial resistance patterns of 86 Shiga toxin-producing *Escherichia coli* (STEC) isolates from beef abattoirs, cattle faeces, raw beef and ready to eat beef products in Gauteng, South Africa. SX: sulphamethoxazole, TC: tetracycline, AM: ampicillin, XL: amoxicillin-clavulanic acid, TR: trimethoprim, TS: cotrimoxazole, CL: chloramphenicol, SM: streptomycin, IP: imipenem, KM: kanamycin, TX: ceftriaxone, AZ: azithromycin, CI: ciprofloxacin, PM: cefepime, NA: nalidixic acid, NI: nitrofurantoin, TZ: ceftazidime**

**Table 3: Phenotypic multidrug resistance (MDR) in Shiga toxin-producing *Escherichia coli* isolates from the beef production chain in Gauteng, South Africa**

No. of isolates	Source	MDR phenotypes*
1	Feedlot	AM, XL, TS, TR, SX, CL, TC, TX, PM
2	Abattoir	AM, XL, TS, TR, SX, CL, TC
1	Feedlot	AM, TS, TR, SX, CL, TC, SM
1	Retail	AM, TS, TR, SX, TC, SM, TX
2	Abattoir	AM, TS, TR, SX, TC, SM
1	Feedlot	AM, XL, SX, CL, TC
1	Feedlot/Abattoir	AM, TS, TR, SX, TC
2	Retail	XL, SX, CL, TC, KM
3	Abattoir	AM, TS, TR, SX
1	Abattoir	AM, XL, TR, SX
1	Feedlot	AM, SX, CL, TC
1	Feedlot	AM, XL, SX, TC
1	Feedlot	SX, CL, TC, SM
1	Abattoir	SX, CL, TC
1	Abattoir	SX, TC, SM
8	Abattoir/Feedlot/Retail	AM, XL, SX
1	Retail	XL, SX, TC
1	Retail	TS, TR, CI

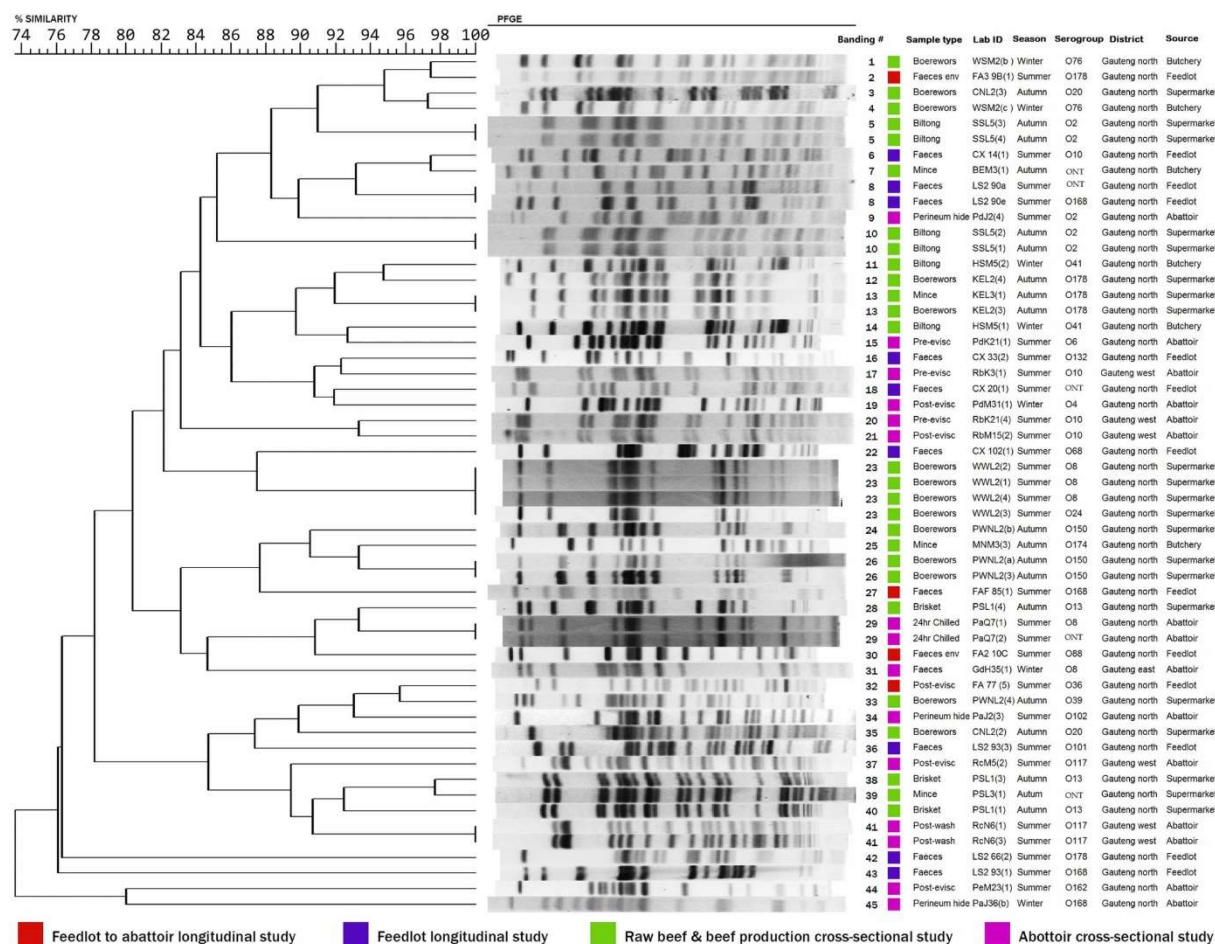
\*MDR phenotypes - AM: ampicillin, XL: amoxicillin-clavulanic acid, TS: cotrimoxazole, TR: trimethoprim, SX: sulphamethoxazole, CL: chloramphenicol, TC: tetracycline, TC: tetracycline, IP: imipenem, TX: ceftriaxone, PM: cefepime, CI: ciprofloxacin

### 3.3. PFGE

For the 55 isolates subtyped by PFGE, the dendrograms showed a high diversity of 45 distinct PFGE banding patterns (Figure 2). This diversity of PFGE patterns was observed in some isolates of the same serogroup that did not cluster together; these included serogroup O178 (feedlot environmental faeces [2], feedlot cattle faeces [42], and supermarket boerewors [12]), two isolates belonging to serogroup O20 (boerewors [3 & 35]) and four O168 isolates (feedlot cattle faeces [8, 27, 43] and abattoir perineum hide swab [45]). The isolates from abattoir [44,

45] and feedlot [43] were distantly related to each other and to 52 other isolates. Furthermore, other serogroups that did not cluster together included O8, O10, O2, O150, O117 and O13. Some patterns were observed in the dendrogram with varying band similarity percentages. At 100% banding similarity, eight band similarity patterns were identified [5,8,10,13,23,26,29,41], except for isolate retail (12)), which showed very similar banding patterns with 13 and 13, but there was a missing band at 28.99 kbp. At 97.5% banding similarity, four closely related patterns were identified in eight isolates (7%): (a) winter butchery boerewors [1] and summer environmental feedlot faeces [2], (b) autumn supermarket boerewors [3] and winter butchery boerewors [4], (c) summer cattle feedlot faeces [6] and autumn butchery mince [7], and (d) autumn brisket [38] and mincemeat [39] from the same supermarket.

Analysis of the PFGE patterns at 93% similarity banding pattern showed that feedlot cattle [6] and butchery mince [7] isolates were closely related to feedlot cattle [8 & 8], and all four isolates related to abattoir perineum hide swab [9]. At 91% similarity banding pattern supermarket biltong [5 & 5] were closely related to four isolates recovered from: butchery-boerewors [1], environmental feedlot faeces [2], and supermarket boerewors [3]. , and butchery-boerewors [4]. At 84.5% band similarity percentage or greater, analysis revealed that most of the clades belonged to isolates from different sources (Figure 2).



**Figure 2: Dendrogram of pulsed-field gel electrophoresis (PFGE) cluster analysis of 55 Shiga toxin-producing *Escherichia coli* (STEC) isolates from different sources in the beef production chain in Gauteng, South Africa.**

## 4. DISCUSSION

Risk characterization of foodborne STEC isolates has shown that the presence of *stx*<sub>2</sub>+*eaeA* gene combination is important in the likelihood of developing HUS and with severe clinical symptoms (FAO/WHO, 2019). In this study, *stx*<sub>1</sub> had the highest prevalence (24%) followed by *stx*<sub>1</sub>+*stx*<sub>2</sub> (19%) and then *stx*<sub>2</sub> (17%). Although with higher frequencies, similar trend was observed in Michigan State, USA from STEC strains isolated from cattle faeces from commercial feedlots and human clinical isolates (Shridhar et al., 2017). Also, in Brazil, *stx*<sub>1</sub> had the highest prevalence in cattle (Gonzalez and Cerqueira, 2020). However, a study of 140

isolates recovered from cattle on five cow-calf operations in two provinces of South Africa, showed a different trend; *stx*<sub>1</sub>+*stx*<sub>2</sub> had the highest prevalence (61.4%), followed by *stx*<sub>2</sub> (34.3%) and then *stx*<sub>1</sub> (4.3%) (Karama et al., 2019). Furthermore, the detection of virulence genes at every stage of the beef production chain underscores the public health importance of the pathogen in Gauteng, South Africa.

The prevalence was highest in the feedlot (34 isolates), followed by the abattoir (27 isolates) and retail (24 isolates). This is supported by numerous published reports from Europe, Australia and the United States (Smith et al., 2010; Lammers et al., 2015; Jaakkonen et al., 2019) emphasizing the risk of cattle carriage in the beef chain. The *eaeA* gene was detected in six different combinations and 70% (14/20 isolates) was observed in the faeces of feedlot cattle, which highlights the potential for contamination of the environment, because STEC can survive for extended periods in the agricultural environment, including in soil (García et al., 2010). However, lower virulence combinations of *eaeA* genes have been reported; 5.11% in South Africa (Montso et al., 2019), 26.92% in Iran, (Dastmalchi et al., 2012) and 4% reported in Malaysia (Perera et al., 2015). STEC contaminated faecal materials can also be washed into water sources following heavy rainfall (Heiman et al., 2015), and this poses a great public health risk for humans (FAO/WHO, 2019).

Notably, twelve STEC isolates (14%) exhibited a 98% to 100% susceptibility to nine antimicrobial agents (nalidixic acid, ceftazidime, nitrofurantoin, imipenem, kanamycin, ceftriaxone, azithromycin, ciprofloxacin and cefepime), which is encouraging because these antimicrobial agents are critically important in human medicine (WHO, 2017). Furthermore, we observed a 100% and 99% susceptibility to nalidixic acid (quinolone) and ciprofloxacin (fluoroquinolone), respectively. Though a rare observation, a similar trend was reported in Rwanda for *E. coli* isolates recovered from faeces of livestock including cattle (Manishimwe et al., 2021), and Hang et al., (2019) also reported fluoroquinolone resistance of *E. coli* isolated



from Calves in Vietnam. More so, quinolone resistance for *E. coli* in low- and middle-income countries (LMIC) range between 20 to 60% (van Boeckel et al., 2020). Considering the *E. coli* genome dynamics and plasticity, which promotes the integration of virulence and/or resistance genes and has the capacity to transform it into a novel hybrid strain (Adefisoye and Okoh, 2016; Lacher et al., 2018). This infrequent occurrence of STEC isolate susceptibility to ciprofloxacin and resistance to nalidixic acid could have resulted from acquired mutated *gyrA* and *parC* genes (Dellgren et al., 2019; Rodríguez-Martínez et al., 2016). Hence, we recommend further characterization of quinolone resistance genes including *qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS* and *aac(6')-ib* (Dellgren et al., 2019; Hang et al., 2019). Overall, our results agree with the findings of Iweriebor et al., (2015), who reported antimicrobial susceptibility to quinolones, fluoroquinolones and aminoglycosides in a study of O157 STEC isolates from dairy cattle farms in the Eastern Cape, South Africa.

*E. coli* strains from ruminant faeces serve as sentinels to track the risk of AMR among Gram-negative bacterial populations and to determine the prevalence of acquired genes in environments due to human activities, including beef production (Cameron and McAllister, 2016). Consequently, the 35% MDR observed in this study may reflect the degree of AMR presence and persistence in the study area, which could be an indicator of levels of antimicrobial use in livestock in South Africa.

STEC isolates exhibited resistance to major antimicrobial classes, including sulphonamides (sulfamethoxazole [92%]), followed by tetracyclines (tetracycline) and penicillins (ampicillin and amoxicillin-clavulanic acid). Our results are consistent with the findings of Eagar et al., (2012), who reported the intensive use of sulphonamides as growth promoter additives in feed premixes and water medications in South Africa. The high prevalence of resistance to sulfamethoxazole (sulphonamides) in our study may be explained, in part, by this practice and should be a source of concern given that this class of antimicrobial agent is widely used for

clinical intervention in both humans and in food-producing animals and is therefore of therapeutic significance (Landers et al., 2012).

In other countries, resistance of STEC to ampicillin and tetracycline in livestock and livestock products has been reported in Egypt (Ahmed and Shimamoto, 2015) and Mexico (Amézquita-López et al., 2016). In South Africa, higher prevalence of resistance to ampicillin (95%), tetracycline (97%), amoxicillin/clavulanate (84%), and trimethoprim/sulfamethoxazole (84%) have been reported for isolates from the faeces of dairy cattle (Iweriebor, 2015). Hence, this could be an evolving public health threat, given that South Africa is ranked with China, Brazil and India as hotspot countries for AMR, i.e., countries with more reports of AMR compared with other developing nations (Van Boeckel et al., 2015). These results underscore the public health implication of STEC contamination in the beef production chain as an important risk factor for disease and for development of acquired AMR (FAO/WHO, 2019).

Analysis of PFGE DNA fingerprints revealed a high diversity of 45 distinct PFGE patterns among 55 non-O157 STEC, which provided relevant molecular epidemiological information on the genomic diversity of non-O157 STEC strains in the beef production chain in Gauteng. Cattle are known to shed a variety of STEC strains, which may be carried along the beef production chain (Buncic et al., 2014; Nastasijević et al., 2014). In our study, the PFGE was useful for epidemiological investigation and has been used to track foodborne bacterial pathogens along the food production chain (Peters, 2009; Lytsy et al., 2017).

At 100% band similarity, we observed eight PFGE-related patterns for 16 isolates originating from the same location and source but from different stages of the beef production chain. These suggest that once a pathogen is established at any production stage (farm, abattoir or retail processing) it may result in within-production-stage transmission (Browne et al., 2018). This suggestion is strengthened by studies that have demonstrated within-pen transmission indicated by observed low variability of STEC strains isolated from calves from the same pen (Baltasar

et al., 2014; Browne et al., 2018) due to re-circulation of the same strain. Similarly, at the 97.5% band similarity we observed eight PFGE-related patterns for eight isolates originating from different stages along the beef production chain. These data provide evidence of epidemiological lineage, implying horizontal transmission of STEC strains along the beef production chain.

Furthermore, most isolates, regardless of source, were predominantly clustered within the 84 to 94% band pattern similarity, suggesting horizontal transmission, which represents an epidemiological link within the Gauteng province. Jackson et al., (2000) demonstrated that differing patterns do not necessarily mean that strains are unrelated. Hence, these band similarity patterns (84 to 94%) provide additional evidence of geographical clustering and variation of STEC serogroups (Hughes et al., 2006). Studies have demonstrated geographical clustering of non-O157 STEC serogroups such as serogroup O80 that was reported to be geographically clustered in eastern France in 2013/14 (Ingelbeen et al., 2018).

## **5. CONCLUSION**

STEC strains isolated along the beef production chain in Gauteng exhibited high heterogeneity. This high variability confirmed by the PFGE subtyping technique and the differences in serogroups, virulence genes and antimicrobial profiles demonstrate the presence of a diverse but related STEC population in the beef production chain, suggesting that there may be no dominant serogroup of STEC in Gauteng. There is also evidence of horizontal transmission and STEC strain recirculation along the beef production chain within the province. The five clinically important serogroups (O8, O174, O2, O20 and O117) should be routinely monitored, and the presence of the *stx<sub>2</sub>+eaeA* virulence gene combination in 8% of the STEC isolates is an indication of the potential health risk to humans. The high prevalence of resistance to sulfamethoxazole and MDR, may reflect the over-use of antimicrobial agents in the livestock

industry. There is need for active epidemiologic surveillance and monitoring in public health laboratories in South Africa.

## **DECLARATIONS**

Declarations of interest: none

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