

1 **Prevalence and distribution of antimicrobial resistance determinants of**
2 ***Escherichia coli* isolates obtained from meat in South Africa**

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27 Abstract

28 *Objective:* This study aimed to characterise antibiotics resistance of *Escherichia coli* isolates
29 from the formal meat sector (FMS) and informal meat sectors (INMS).

30 *Method:* A total of 162 and 102 *E. coli* isolates from the FMS, and INMS respectively were
31 isolated by standard culture-based, and biochemical reactions. The isolates were further
32 confirmed by polymerase chain reaction (PCR). The disc diffusion method was used to
33 screen for antimicrobial susceptibility against 19 different antibiotics. The presence of class
34 1-2 integrons in each *E. coli* isolates was assessed using 3'-CS and 5'-CS regions specific
35 primers.

36 *Result:* Among the 19 antimicrobials, resistance to tetracyclines, aminoglycosides,
37 cephalosporins, and nitrofurans were found to be more frequent than carbapenems and
38 phenicol with a noticeable increase in the number of multi-drug resistance ranging from three
39 to ten antimicrobials. A total of 20 resistance determinants were assessed with their
40 prevalence and distributions obtained as follows for FMS and INMS respectively;
41 [aminoglycosides: *aadA* (40.6%; 31.9%), *aacC2* (21.4%; 31%), *aphA1* (20.8%; 15.1%),
42 *aphA2* (37.7%; 18.9%) and *strA* (6.5%; 9.4%)], [β -lactams: *ampC* (20%; 45%), *blaTEM*,
43 (4.4%; 13.3), and *blaZ* (8.9%; 2.2%)], [Chloramphenicol: *catI* (1.7%; 1.7%), and *cmIAI*
44 (1.7%; 1.7%)] and [tetracyclines: *tetA* (7.7%; 15.4%), *tetB* (11.5%; 24%), and *tetM*, (1.9%;
45 8.7%)], and [sulfonamides: *sul1* (22.2%; 26.7%), *sul2* (17.8%; 6.7%)].

46 *Conclusion:* Multiple antibiotic resistance (MAR) indexes ranged from 0.2 - 0.5. The results
47 reveal a high prevalence of multidrug-resistant *E. coli* isolates and resistance determinants
48 suggesting that consumers and handlers of such meat are at risk of contracting antibiotic
49 resistant *E. coli*-related foodborne disease.

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51 **Keywords:** Abattoir; antimicrobial resistance; Eastern Cape Province; food safety; meat

52 1 Background

53 Meat is a high-risk food, and many studies have shown a causal relationship between meat
54 consumption and disease outbreaks. Meat-related foodborne diseases frequently occur due to
55 the consumption of *Escherichia coli* contaminated raw or poorly processed meat. The United
56 States, Center for Disease Control and Prevention (CDC) estimates for the year 2011 that one
57 in six or forty-eight million people are infected with a foodborne illness each year, resulting
58 in 3000 deaths. Of this number, *E. coli* O157: H7 caused an estimated 73480 illnesses each
59 year, resulting in more than 2000 hospitalisations and 60 deaths [1].

60 Even though raw food hygiene and food safety epidemiology is still in its infancy in Africa,
61 foodborne disease (FBD) is common in developing countries. For instance, 10200 cases of
62 Shiga toxin *E.coli* (STEC) related FBD was reported in WHO subregion AFR D and E circa

63 2012 [2]. The actual prevalence of food-borne infections is difficult to determine, primarily
64 because only a small percentage of incidence is officially reported. Even when cases of
65 foodborne infections are reported, only in a limited number is the aetiology determined [3].

66 Antibiotics play a vital role in the treatment and management of bacterial infections, leading
67 to a reduction in morbidity and mortality of both human and animal patients. However, the
68 misuse of antibiotics in agriculture, veterinary and medical enterprises drives the selection of
69 antibiotic-resistant bacteria that resist and overcome the action of the antibiotic.
70 Approximately, 80% of all antibiotics used worldwide are in agriculture and aquaculture [4].
71 In livestock husbandry, antibiotics are used for prevention of infection or the simultaneous
72 treatment of healthy and sick animals in a group during an outbreak of disease. It can further
73 be used as antimicrobial feed additives (AFAs) for growth promotion and performance in
74 production animals [4,5].

75 In 2006, the European Union (EU) prohibited the use of AFA for growth promotion in swine,
76 cattle, poultry, and rabbits. Similarly, the US Food and Drug Administration opted for the
77 voluntary phasing out of antibiotics for growth promotion (AGP) in animals in the USA in
78 December 2013 [4,6]. However, in many developing countries, no such bans or plans to
79 phase out AGPs have been put in place [4] and yet many of these countries bear the heavy
80 burden of foodborne diseases caused by resistance pathogens.

81 Although epidemiological surveillance has established an association between the usage of
82 antibiotic and antimicrobial resistance [7], South Africa registered sixty-four antimicrobial
83 products, representing nineteen active pharmaceutical ingredients registered as in-feed mixes
84 for growth promotion in the years 2002-2004. This included WHO-banned antimicrobials
85 such as virginiamycin, spiramycin, tylosin, and bacitracin [7]. Meaning that South Africa still
86 permits the use of these compounds as antibiotic feed additives, hence, it is no wonder that

87 AFAs constituted two-thirds of all antimicrobials sold for animal use over these three years
88 [4,5].

89 South Africa faces the challenge of scarcity of veterinary expertise needed to provide
90 professional veterinary care, surveillance and monitoring of antimicrobial use by farmers.
91 The shortage of veterinary surgeon in the country was one of the reasons the Fertilisers,
92 Farm, Feeds, Agricultural Remedies and Stock Remedies Act (Act 36 of 1947) allows
93 farmers to purchase and use some antimicrobial agents without needing to produce a
94 veterinary prescription. As a result, farmers can purchase and administer many of the
95 prescribed veterinary antibiotics since they can be obtained as over-the-counter (OTC) stock
96 remedies [8]. It is then no surprise that in 2002-2004, 72% of antimicrobials used in animals
97 in South Africa were permitted by the Stock Remedies Act.

98 *Escherichia coli* is a ubiquitous gut microorganism which forms part of the natural flora of
99 the gastrointestinal system. Its ability to acquire both resistant determinants and virulence
100 factors has been acknowledged by many researchers [9]. Some *E.coli* strains are highly
101 pathogenic and are categorised based on their virulence into different pathogroups. These
102 pathogroups include enterotoxigenic *E. coli* (ETEC); enteropathogenic *E. coli* (EPEC); Shiga
103 toxin-producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli*
104 (DAEC); and enterohemorrhagic *E. coli* (EHEC); a sub-class of enteroinvasive *E. coli*
105 (EIEC); neonatal meningitis *E. coli* (NMEC), enteroaggregative *E. coli* and uropathogenic *E.*
106 *coli* (UPEC) [10]. The pathogenicity of each pathotype depends on its ability to adhere,
107 colonise, and invade the host's cells system causing cell death and apoptosis. The secretion
108 and transportation of cell surface molecules, siderophore formation, and toxins are other
109 methods of establishing virulence in the host [1].

110 Foodborne diseases (FBD) associated with resistant *E. coli* has reached an alarming
111 proportion and remains a global public health crisis [11]. The transfer of resistance to enteric
112 and commensal bacteria enhancing pathogenicity when consumption of contaminated food
113 and water occur is even a bigger problem. The emerging resistance to WHO classified
114 critically important antimicrobial such as carbapenems, extended-spectrum cephalosporins
115 (ESCs), aminoglycosides and fluoroquinolones (FQs) among Enterobacteriaceae remains
116 worrisome [12]. Studies' comparing the AMR in the formal and informal meat sectors are
117 limited. Hence, constant surveillance of the resistance profile of the bacteria is a useful early
118 warning epidemiological indicator. This study aimed to determine the phenotypic and
119 genotypic profile of antimicrobial resistant *E. coli* isolates obtained from abattoir and
120 slaughter points the formal and informal meat sectors in the Eastern Cape Province of South
121 Africa.

122 **2 Material and methods**

123 **2.1 Sample site and collection**

124 Samples from the formal meat sector (FMS) were collected at three high throughput abattoirs
125 located in the East London (HT1), Queenstown (HT2) and Port Elizabeth (HT3) from the
126 year 2015 to 2016. During the same period (2015-2016) samples were also collected from the
127 informal meat sector (INMS) in different towns such as Alice town (AT), King Williams'
128 town (KWT), and Cala town (CT) (Figure 1). Carcasses from the informal sector were
129 slaughtered for traditional use, home use, and the informal meat market. A total of 83 and 35
130 carcasses were sampled in the FMS and INMS respectively by swabbing the rump, neck,
131 brisket, and flank areas. Sterile throat cotton swab moistened with peptone water was used to
132 swab a 100cm² carcass surface of beef, mutton, and pork. All samples were transported in a
133 cooler box to the laboratory for the detection and confirmation of the presence of *E. coli*. In

134 total, 332 and 140 samples were collected from the formal and informal meat sector
135 respectively. A total of 162 and 102 *E. coli* isolates were confirmed by molecular method
136 (PCR) in formal and informal meat sector respectively (Figure 2). All the confirmed (n = 162
137 + 102) confirmed isolates were stored in glycerol for further antimicrobial susceptibility
138 testing.

139 **2.2 Isolation and identification of *Escherichia coli***

140 All the swabs from all sampling sites were inoculated into tryptic soy broth (TSB) and were
141 incubated for 24 hours at 37°C. Samples from the tryptic soy broth were then inoculated onto
142 Eosin Methylene Blue agar (EMB) on different plates and incubated for 24-48 hours at 37°C.
143 The EMB is a selective enrichment media and differential for *E. coli*. Single pure green
144 metallic sheen colonies characteristic of *E. coli* on EMB were confirmed as presumptive
145 isolates and stored in 30% glycerol awaiting further analysis.

146 **2.3 DNA extraction**

147 The presumptive *E. coli* isolates previously stored in glycerol were resuscitated and plated on
148 EMB plates. Five distinctive colonies were picked from the EMB plates and inoculated into
149 the nutrient broth and incubated for 48 hours at 37°C. DNA was extracted by the boiling
150 method [13]. Briefly, 1 ml of broth solution containing *E. coli* was transferred to an
151 Eppendorf tube and centrifuged (Thermo Fisher Scientific, Germany) for 15 mins at a speed
152 of 13000 rpm. The supernatant was discarded, and the pellet was retained. Again another 1
153 ml of the broth was added and centrifuged at the same speed and duration; this was done five
154 times to obtain a sizeable pellet. To wash the pellet; 200 µL of distilled water was added to
155 the pellet and discarded. Again a volume of 200 µL of distilled water was added to the
156 washed pellet, vortexed and centrifuged at a speed of 13000 rpm for 5 mins and the
157 supernatant was discarded. The pellet was then placed on AccuBlock™ Digital Dry Baths

158 (Labnet International, USA) at 100°C for 15 mins to lyse the cell. Cell debris was removed
159 by centrifugation at 13000 rpm for 10 mins while the supernatant was stored as the DNA
160 template.

161 **2.4 PCR confirmation of *E. coli* isolates**

162 Confirmation of presumptive *E. coli* isolates was by Polymerase chain reaction (PCR) in a
163 total volume of 25µL containing 5.0µL of the DNA template, 5.5µL nuclease-free water,
164 12.5µL master mix, 1.0µL forward primer, and 1.0µL reverse primer. The *UidA* primers
165 were used for PCR testing of the bacterial isolates. *Escherichia coli* ATCC 25922 served as
166 the positive control strain [14]. The PCR condition is as follows: Initial denaturation at 94°C
167 for 2 mins followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1
168 min and extension at 72°C for 1 min. A final extension at 72°C for 2 mins. Holding was at
169 4°C (Table 1).

170 **2.5 Antibiotic susceptibility profiles of *E. coli* isolates**

171 The antimicrobial resistance profiles of confirmed isolates were determined using the Kirby–
172 Bauer disk diffusion method on Mueller–Hinton agar [14]. Isolates were inoculated onto
173 nutrient agar and incubated at 37 °C for 24 hours. A single colony was picked from the
174 nutrient agar plate and suspended into 0.9 saline water and adjusted to give a reading of 0.5
175 McFarland turbidity standard. A 0.1 ml volume of the 0.5 McFarland suspension was
176 swabbed evenly in at least three directions on the surface of a Mueller–Hinton agar plate. The
177 surface of each plate was left to dry up, and then antimicrobial disks for each antimicrobial
178 were placed at a specific place on the surface of the agar.

179 The plates were incubated lid side up at 37 °C for 24 hours. The zone of inhibition was
180 recorded by measuring the size of the zone of inhibition around the disk. Isolates were
181 classified as being resistant, intermediate and sensitive based on the Clinical and Laboratory

182 Standards Institute guidelines [15]. Isolates which fell in the intermediate category were
183 reclassified as being resistant [16].

184 Based on the CLSI guidelines, the following antibiotics were used for the antibiotic
185 susceptibility test: Cotrimoxazole (25µg), Ciprofloxacin (5µg), Norfloxacin (10µg),
186 Amoxicillin (30µg), Ampicillin (25µg), Tetracycline (30µg), Gentamicin (10µg),
187 Streptomycin (300µg), Kanamycin (30µg), Neomycin (10µg), Ceftriaxone (30µg),
188 Cefotaxime (30µg), Ceftazidime (10µg), Imipenem (10µg), Meropenem (10µg), Ertapenem
189 (10µg), Doripenem (10µg), Chloramphenicol (30µg), Nitrofurantoin (300µg).

190 **2.5 Detection of antimicrobial resistance genes**

191 Specific primer sequences for the various resistance gene coding the phenotypic resistance of
192 isolates observed were subjected to PCR assay as previously described [13]. Table 2
193 summarises the details of the process of gene sequencing. For cycling, a Bio-rad thermal
194 cycler (Bio-Rad Mycycler, USA) was used. For antimicrobial classes such as sulphonamides,
195 beta-lactams, tetracyclines, aminoglycosides, and phenicols, isolates were tested for the
196 possession of various genotypic resistance determinants e.g. *aac(3)-IIa (aacC2)*, *aph(3)-Ia*
197 (*aphA1*), *aph(3)-IIa (aphA2)*, *aph(3)-Ia (aphA1)*, *aph(3)-IIa (aphA2)*, *aadA*, *strA*, *blaTEM*,
198 *blaZ*, *ampC*, *cat1*, *cat2*, *cmlA1*, *sull1*, *sul2*, *tetA*, *tetB*, *tetC*, *tetD*, and *tetM*.

199 **2.6 Gel electrophoresis**

200 The amplified products were visualised by ethidium bromide staining after gel
201 electrophoresis of 10 µL of the final reaction mixture in 1.5% agarose for 45 mins.

202 **2.6 Statistical analysis**

203 The data was captured in Microsoft Excel® (Microsoft Corporation, USA) and analysed
204 using SPSS software (Version 24. IBM SPSS Inc, United States). The data were analysed to
205 test for correlation between antibiotics resistance properties of *E. coli* isolate in the formal

206 and informal meat sector. Statistical significance was set at P value < 0.05. Multiple
207 antibiotics resistance index was calculated using the formula:

$$208 \text{ MARI} = a/b$$

209 Where (a) is the aggregate antibiotic resistance score of all isolates from the sample, (b) is the
210 total number of antibiotics used [17]. A MAR index ≥ 0.2 indicates the high-risk environment
211 where antibiotics are often used [13].

212 **3 Results**

213 **3.1 Isolation of *Escherichia coli***

214 From 83 carcasses that were sampled in the present study from formal meat sector, 322
215 samples were obtained. The 322 samples then yielded 162 confirmed *E. coli* isolates, of
216 which 57, 44 and 61 isolates originated from HT1, HT2, and HT3 respectively (Figure 2).
217 With regard to the informal meat sector, 35 carcasses were sampled, and these gave us 140
218 samples. The 140 samples then yielded 102 confirmed *E. coli* that included 27, 46, and 29
219 isolates from AT, KWT, and CT respectively.

220 **3.2 Antimicrobial Susceptibility Testing**

221 Antimicrobial resistance rate for *E. coli* isolates for the formal meat sector was as follows
222 (Table 3): streptomycin 54.9% (89/162); ceftriaxone 54.9% (89/162); tetracycline 43.8%
223 (71/162); nitrofurantoin 40.1% (65/162); neomycin 35.2% (57/162); amoxicillin 22.8%
224 (37/162); ceftazidime and chloramphenicol 21.6% each (35/162); and kanamycin 20.4%
225 (33/162). The proportion of drug resistance in isolates from the informal meat sector was as
226 follows (Table 3): streptomycin 48.0% (49/102); tetracycline 32.4% (33/102); neomycin
227 30.4% (31/102); chloramphenicol 24.5% (25/102); gentamicin 22.5% (23/102); imipenem
228 21.6% (22/102); ceftriaxone 20.6% (21/102); kanamycin 19.6% (20/102); and cotrimoxazole
229 15.7% (16/102). Multiple antibiotic-resistant phenotypes (MARPs) pattern for isolates from

230 the formal meat sector ranged from 1-5 (MARI, 0.2-0.5) and MARPs for the informal meat
231 sector ranged from 2-15 (MARI, 0.2-0.5) (Table 4).

232 **3.3 Antimicrobial resistance genes and pattern of resistance**

233 Aminoglycoside resistance determinants were the most common (Table 5) and were as
234 follows for isolates from the formal sector: streptomycin (*aadA*: 40.6%); kanamycin (*aphA1*:
235 20.8; *aphA2*: 37.7%); gentamycin (*aacC2*: 21.4%). Other resistant determinants that were
236 common include the ones for cotrimoxazole (*sul1*: 22.2%) and (*sul2*: 17.8); ampicillin
237 (*ampC*: 20%); and tetracycline (*tetB*: 11.5%).

238 Resistance determinant found in the informal sector was streptomycin (*aadA*: 31.9%);
239 kanamycin (*aphA1*: 15.1%) and (*aphA2*: 18.9%); neomycin (*aphA1*: 14.8%); and gentamycin
240 (*aacC2*: 31%). Others were cotrimoxazole (*sul1*: 26.7%); amoxicillin (*blaTEM*: 13.3%);
241 ampicillin (*ampC*: 45%); and tetracycline (*tetA*: 15.4%; *tetB*: 24%; *tetD*: 12.5%).

242 The number of genotype resistance determinants pattern ranged from 2-4 for the various
243 antibiotic genes tested. In the formal sector, 1-4 isolates were found to have multiple genes
244 coding for resistance determinants. However, in the informal sector, 1-12 isolates possessed
245 multiple genetic components for antimicrobial resistance determination (Table 6).

246 **4 Discussion**

247 Livestock is recognised as a primary reservoir of various pathotypes of *Escherichia coli*,
248 which have been epidemiologically linked to many incidences of meat-related food-borne
249 diseases [18]. Hence, monitoring the microbial quality of meat using indicator organisms
250 such as *E. coli* is of medical and veterinary importance [19]. The development of
251 antimicrobial resistance among pathogens that impact human and animal health further
252 buttresses the need for intensified surveillance. Thus *E. coli* due to its existence in the

253 gastrointestinal tract of animals and its ability to acquire antimicrobial resistance has been
254 designated as a sentinel organism in antimicrobial resistance surveillance programs
255 worldwide [20].

256 In the current study, meat processed in the formal (49%) and informal (73%) harboured *E.*
257 *coli* (Figure 2). These results suggest that contamination of meat does occur during slaughter,
258 and thus highlights the quality of meat processed in both the formal and informal meat
259 sectors. The result of this study agrees with the 74%, 57.9% and 43% prevalence of *E. coli*
260 found from beef in South Africa, Colombia and Burkina Faso respectively [21–23]. However,
261 a low prevalence of 1-4% and 2.8% of *E. coli* was reported in the city of Graz, Saudi Arabia
262 and Amatole district of the Eastern Cape Province, South Africa respectively [24,25]. Both
263 studies mainly focused on *E. coli* O157: H7, hence the differences in both studies could
264 easily be explained given the very low levels of prevalence of *E.coli* observed compared to
265 what we observed in the present study.

266 The authors expected meat from the formal sector to be of high microbial quality. However,
267 results reported here indicated that just under half (49%) of the carcasses from the formal
268 sector were positive for *E. coli*. Even though this was lower than the 73% from the informal
269 market, it can be considered to be very high. Contamination of carcass at abattoirs could be a
270 direct consequence of dirty hides or airborne pathogens, contaminated knife, aprons hands
271 and other cutting tools [26]. On the other hand, animal slaughter in the informal sector is
272 performed in an unhygienic, unorganised and unregulated environment. This explains why a
273 higher level of contamination was observed among carcasses from the informal sector
274 compared to those from the formal sector. In one study of meat safety knowledge among
275 butchers during traditional meat slaughter, many meat handlers during traditional meat
276 slaughter demonstrated moderate knowledge of meat safety rules [27]. However, in practice

277 this is hardly true given that meat from the informal market is commonly associated with a
278 greater risk of food poisoning [28].

279 Globally, the bacteriological safety of meat is a pressing public health concern. Slaughter and
280 dressing with personnel practices carry the highest value when analysing the risk of
281 contaminating the carcass. Thus a breach in hygiene protocol could lead to the introduction of
282 microorganisms at the production, processing, and consumption of meat and meat products
283 [27], hence the need for a risk-based food safety system.

284 In the formal meat (FMS), profoundly high phenotypic resistance was observed for
285 streptomycin (54.9%), ceftriaxone (54.9%), tetracycline (43.8%) and nitrofurantoin (40.1%),
286 all of which are antimicrobials commonly used in human medicine. On the other hand,
287 antimicrobial resistance in the informal meat (INMS) sector tended to be highest for
288 streptomycin (48.0%), neomycin (30.4), tetracycline (32.4%), chloramphenicol (24.5%),
289 imipenem (21.6%) and ceftriaxone (20.6%). Although studies' comparing the AMR in the
290 formal and informal meat sector are limited, results of the present study are consistent with
291 available studies on AMR in the meat sector that reported a sustained prevalence of AMR to
292 streptomycin and tetracycline as well as the emerging AMR to cephalosporins [19].

293 The possible reason for the variability in the proportions of AMR in the FMS and INMS
294 could be sample size, hygiene management systems, and the application of antibiotics for
295 prophylaxis and metaphylaxis in livestock farms in both meat sectors. The other reason could
296 be the co-selection of resistant determinant and co-resistance of the *E. coli* isolates. The
297 simultaneous resistance to penicillin, streptomycin, tetracycline, erythromycin, kanamycin,
298 and virginiamycin has been reported in studies conducted elsewhere [29–31]. A similar co-
299 selection of sulphonamide resistance genes was reported in chickens treated with
300 streptomycin [32]. Moreso, a Canadian study found a predominant pattern of AMR with

301 extended-spectrum cephalosporin (ESC)-resistant *E. coli* strains, with co-resistance to
302 streptomycin, cefoxitin, trimethoprim-sulfamethoxazole, sulfisoxazole, ampicillin,
303 amoxicillin/clavulanic acid, chloramphenicol, and tetracycline [30]. Also, in an extensive
304 study of *E. coli* isolates from various countries authors observed that nearly 75% of
305 ampicillin-resistant *E. coli* isolates were also resistant to streptomycin and tetracycline [33].
306 Hence, the authors of that study suggested that the resistance genes for these drugs are linked
307 on plasmids.

308 The prevalence of streptomycin resistance in this study could be linked to its extensive use
309 for the treatment of bacterial infections of plants and animals [34,35]. Generally,
310 aminoglycosides resistance is mediated by aminoglycoside-modifying enzymes, including
311 acetyltransferases and nucleotidyltransferases, aminoglycoside phosphotransferases, and 16S
312 rRNA methylases, all of which have been reported in Enterobacteriaceae [36]. The increasing
313 prevalence of resistance against streptomycin has led to its designation as a critical
314 epidemiological marker to indicate the likelihood of multidrug-resistance (MDR) in
315 pathogens [34]. Streptomycin resistance is frequently mediated by *aadA* genes, which are
316 typically present on integrons causing streptomycin adenylation [34,37]. Thus, it is not
317 surprising that the *aadA* gene and to a lesser extent the *strA* gene were the main genetic
318 element of resistance observed in the present study (Table 6.5).

319 Cephalosporins such as ceftriaxone, cefotaxime, and ceftazidime are used in food-producing
320 animals, and this imposes greater selection pressure for the development of extended-
321 spectrum β -lactamases (ESBL)-producing and multiple-antimicrobial-resistant *E. coli*.
322 ESBLs are the main contributors to extended-spectrum cephalosporin (ESC) resistance in *E.*
323 *coli* and transfer resistance to cephalosporins with an oxyimino side chain [38]. Resistance to
324 extended spectrum cephalosporinases (ESCs) in *E. coli* has been linked with the extended-

325 spectrum β -lactamases (ESBLs) and plasmid-mediated Ambler class C cephamycinases [39].
326 Cephalosporin resistance has also been associated with extensive use of antibiotics in clinical
327 practice. It is highly possible that apart from cross-contamination of the carcass with animal
328 faeces during slaughter, that many *E. coli* isolates in the INMS could originate from humans.
329 Therefore ceftriaxone resistance in FMS and INMS could be due to the inherent danger of the
330 spread of mobile genetic element of resistance through horizontal gene transfer.

331 In South Africa, Stock Remedies Act, 1947 permits tetracycline to be purchased over the
332 counter (OTC) without a veterinary prescription [40]. Therefore tetracycline resistance in
333 FMS and INMS could be a direct consequence of its widespread use in the treatment of
334 bacterial infection and tick-borne diseases in livestock [5]. Ticks are endemic in the province
335 where the study was conducted. Hence farmers routinely treat their animal with tetracycline
336 prophylactically to prevent disease outbreak. Besides, low doses of tetracycline have been
337 used to control weaning diarrhoea and also included in the feed as antibiotic feed additives
338 (AFAs) for growth promotion [5]. The results observed in this study are in agreement with
339 studies done in Ethiopia, Iran, and Pakistan [41–44]. Meanwhile, our findings contradict
340 findings of earlier studies done in South Africa and Poland that reported lower prevalences
341 [25,29].

342 Although the *tet A, B, C, D* and *M* genes were observed in both FMS and INMS, the burden
343 of resistance was more in the INMS. The high prevalence of *tet* in INMS suggests the
344 therapeutic overuse or misuse of tetracyclines by communal farmers who happen to be the are
345 the main suppliers of animals slaughtered on the INMS. The result of the study were
346 expected, considering that over 70% of antibiotics used in livestock production in South
347 Africa can be purchased over the counter [4].

348 Although the use of chloramphenicol in veterinary medicine and aquaculture has been banned
349 worldwide, ampicillin, cotrimoxazole and other antimicrobials are still commonly used in
350 livestock production in South Africa for treatment, prophylaxis and growth promotion
351 purposes [4,5]. The injudicious use of these drugs may exert selective pressure sustaining the
352 emergence of resistant bacterial strains. Furthermore, co-selection of multiple resistance
353 mechanisms through the use of various antibiotics is possible because resistance genes for
354 many antimicrobial agents are placed on single conjugative plasmids [45]. Apart from the
355 previously mentioned mechanism of multidrug resistance (MDR), inactivation or enzymatic
356 degradation of antimicrobials and chemical transformation of antimicrobial compounds by
357 glycosylation, adenylation, acetylation, phosphorylation, and hydroxylation have also become
358 steadily more apparent as causes of MDR [37]. Some of these mechanisms might be
359 responsible for resistance observed among pathogens studied in this study, and could also be
360 responsible for the resistance to nitrofurantoin which is not commonly used in veterinary
361 medicine in South Africa.

362 **5 Conclusion**

363 This study revealed a high burden of resistance against important antimicrobials such as
364 streptomycin, neomycin, ceftriaxone, chloramphenicol, and tetracycline including imipenem
365 and meropenem. Genes encoding cephalosporin resistance are commonly situated on self-
366 transmissible plasmids which may be promiscuous and capable of disseminating into a broad
367 range of microbiota. Furthermore, resistance of *E. coli* isolates to antibiotics of choice in
368 human therapy such aminoglycoside, phenicols, carbapenems, and cephalosporins pose a
369 grave danger for success in human chemotherapy. Resistance in nitrofurantoin, a drug not
370 commonly in use in South Africa, suggests that factors other than selective pressure must
371 have an impact on the emergence of resistant *E. coli*.

372 In this study, only *bla*TEM and *bla*Z genes were tested, but the possibility of the *E. coli*
373 isolates harbouring other ESBL genes such as *bla*CTX, and *bla*SHV is highly plausible;
374 suggesting that these isolates could potentially be dangerous to public health. Such risk
375 becomes pronounced in compromised food systems as bacteria strains can be transferred to
376 humans via the food chain. Aside the mobilisation of plasmids that can lead to transfer of
377 resistance genes to other Gram-negative and commensal bacteria in the environment, the
378 public health and veterinary concern regarding AMR warrants a sustained and concerted
379 local, regional and international coordinated surveillance and containment system for
380 effective prevention of AMR in food animals. Simple intervention strategies, such as the
381 prudent use of antimicrobials, promoting regular intermittent washing of hand and knife with
382 hot water and soap by slaughterhouse workers and good hygienic practices at the abattoirs
383 and informal slaughterhouses, can have a profound impact on public health.

384 **Abbreviations**

385 DAEC: diffusely adherent *E. coli*; EHEC: enterohemorrhagic *E. coli*; INMS: Informal meat
386 sector; UPEC: uropathogenic *E. coli*; AFA: Antimicrobial feed additives; AGP:
387 Antimicrobial growth promoters; AMR: Antimicrobial resistance; AT: Alice town; CDC:
388 Center for Disease Control and Prevention; CT: Cala Town; EAEC: enteroaggregative *E.*
389 *coli*; EAEC: enteroaggregative *E. coli*; EIEC: enteroinvasive *E. coli*; EMB: Eosin methylene
390 blue; EPEC: enteropathogenic *E. coli*; ESCs: extended-spectrum cephalosporins; ETEC:
391 enterotoxigenic *E. coli*; EU: European Union; FBD: Foodborne disease; FMS: Formal meat
392 sector; FQs: fluoroquinolones; High throughput abattoirs in the East London (HT1),
393 Queenstown (HT2) and Port Elizabeth (HT3); KWT: King William town; MARI: Multiple
394 antimicrobial resistance index; MARPs: Multiple antimicrobial resistance phenotypes;

395 NMEC: neonatal meningitis E. coli; STEC: Shiga toxin-producing E. coli; TSB: Tryptic soy
396 broth; WHO: World Health Organisation

397 **Ethics approval and consent to participate**

398 Ethical approval number MUC351SJAJ01 was obtained from the University of Fort Hare
399 research ethics committee.

400 **Consent for publication**

401 Written consent for publication was obtained from each participating abattoir prior to the
402 microbial survey.

403 **Availability of data and material**

404 The data that support the findings of this study are available from University of Fort Hare
405 repositories. Data are however available from the authors upon reasonable request and with
406 permission of the University of Fort Hare

407 **Competing interests**

408 Authors declare no conflict of interest

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413 **Authors' contributions**

414 IFJ designed and carried out the study, JO edited the manuscript and made useful technical
415 and specialist inputs, EG and VM supervised the study.

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Table 1: Primer sequence and PCR cycling condition of the targeted gene that confirms *E. coli*

Target gene	Primer	Nucleotide sequence (5'-3')	Amplicon size (bp)	PCR cycling condition	References
<i>Uida</i>	Forward	AAAACGGCAAGAAAAAGCAG	147	Initial denaturation at 94°C for 2 mins followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. a final extension at 72°C for 2 mins. Holding was at 4°C.	[46]
	Reverse	ACGCGTGGTTAACAGTCTTGCG			

Table 2: Primers set for antimicrobial resistance gene detection

Antimicrobial class	Primer	PCR primer sequence (5'-3')	Amplicon size (bp)	PCR cycling condition	References
Aminoglycosides	<i>aac(3)-IIa (aacC2)a</i>	F: CGGAAGGCAATAACGGAG R: TCGAACAGGTAGCACTGAG	428	5 mins at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min and a final incubation at 72°C for 5 mins.	[13]
	<i>aph(3)-Ia (aphA1)a</i>	F: ATGGGCTCGCGATAATGTC R: CTCACCGAGGCAGTTCCAT	600	5 mins at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min and a final incubation at 72°C for 5 mins.	[13]
	<i>aph(3)-IIa (aphA2)a</i>	F: GAACAAGATGGATTGCACGC R: GCTCTTCAGCAATATCACGG	510	5 mins at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min and a final incubation at 72°C for 5 mins.	[13]
	<i>aadA</i>	F: GTGGATGGCGGCCTGAAGCC R: AATGCCAGTCGGCAGCG	525	94°C for 4 mins followed by 30 cycles of 94°C for 45 s, 50 °C for 45 s and extension at 72°C for 45 s and final extension for 7 mins	[13]
	<i>strA</i>	F CTTGGTGATAACGGCAATTC R: CCAATCGCAGATAGAAGGC	348	94°C for 4 mins of initial denaturation, followed by 30 cycles of denaturation at 94°C for 45 s, annealing for 45 s at 50°C, extension at 72°C for 45 s and final	[13]

				extension for 7 mins at 72°C.	
Beta-lactams	<i>blaTEM</i>	F: TTTCGTGTCGCCCTTATTC R: CCGGCTCCAGATTTATCAGC	690	94°C for 5 min followed by 30 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), extension (72°C for 90 s) and final incubation at 72°C for 5 mins.	[13]
	<i>blaZ</i>	F: ACT TCA ACA CCT GCT GCT TTC R: TGA CCA CTT TTA TCA GCA ACC	490	94°C for 5 min followed by 30 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), extension (72°C for 90 s) and a final incubation at 72°C for 5 mins.	[13]
	<i>ampC</i>	F: TTCTATCAAMACTGGCARCC R: CCYTTTTATGTACCCAYGA	550	Initial denaturation for 5 mins at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min and a final extension at 72°C for 5 min.	[10]
Phenicols	<i>cat1</i>	F: AGTTGCTCAATGTACCTATAAC C R:TTGTAATTCATTAAGCATTCT GCC	320	5 mins at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min and final incubation at 72°C for 5 mins	[47]
	<i>cat2</i>	F: ACACTTTGCCCTTTATCGTC R: TGAAAGCCATCACATACTGC	543	94°C for 5 mins followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min and a final incubation at 72°C for 5 mins.	[47]
	<i>cmlA1</i>	F: CACCAATCATGACCAAG R:	115	94°C for 5 mins followed by 30 cycles of	[13]

		GGCATCACTCGGCATGGACATG		94°C for 30 s, 50°C for 30 s, and 72°C for 1.5 min and a final incubation at 72°C for 5 mins.	
Sulphonamides	<i>sul1</i>	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	822	Initial denaturation at 94°C for 5 mins, followed by 1 min of denaturation at 94°C, 1 min of annealing at 55°C, 5 min of extension at 72°C for a total of 35 cycles and 5 min of final extension at 72°C.	[47]
	<i>sul2</i>	F: CGGCATCGTCAACATAACC R: GTGTGCGGATGAAGTCAG	625	Initial denaturation for 5 mins at 94°C, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1.5 min and a final extension at 72°C for 5 min.	[47]
Tetracyclines	<i>tetA</i>	F: GCTACATCCTGCTTGCCTTC R: CATAGATCGCCGTGAAGAGG	201	5 mins initial denaturation at 94 °C followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min and a final incubation at 72 °C for 5 mins.	[48]
	<i>tetB</i>	F: TTGGTTAGGGGCAAGTTTTG R: GTAATGGGCCAATAACACCG	359	5 mins initial denature at 94°C followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1.5 min and a final incubation at 72 °C for 5 min.	[48]
	<i>tetC</i>	F: CTTGAGAGCCTTCAACCCAG R: ATGGTCGTCATCTACCTGCC	418	5 mins initial denaturation at 94°C followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1.5 min and a final incubation	[48]

			at 72°C for 5 min.	
<i>tetD</i>	F: AAACCATTACGGCATTCTGC R: GACCGGATACACCATCCATC	300	5 mins initial denaturation at 94°C followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1·5 min and a final incubation at 72°C for 5 mins.	[48]
<i>tetM</i>	F: AGT GGA GCG ATT ACA GAA R: CAT ATG TCC TGG CGT GTC TA	158	5 min initial denaturation at 94°C followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1.5 min and a final incubation at 72°C for 5 min	[48]

Table 3: Antibiotic susceptibility pattern of *E. coli* isolates

Antimicrobial class	Antimicrobials	Disc code	Potency (μg)	Meat sector					
				Formal (n = 162)		Informal (n = 102)		Total (n = 264)	
				R(%)	S(%)	R(%)	S(%)	R(%)	S(%)
Sulphonamides	Cotrimoxazole	TS	25	29(17.9)	133(82.1)	16(15.7)	86(84.3)	45(17)	219(83)
Quinolones	Ciprofloxacin	CIP	5	0(0)	162(100)	11(10.8)	91(89.2)	11(4.2)	253(95.8)
	Norfloxacin	NOR	10	6(3.7)	156(96.3)	2(2)	100(98)	8(3)	256(97)
Beta-lactams	Amoxicillin	A	30	37(22.8)	125(77.2)	8(7.8)	94(92.2)	45(17)	219(83)
	Ampicillin	AMP	25	13(8)	149(92)	7(6.9)	95(93.1)	20(7.6)	244(92.4)
Tetracyclines	Tetracycline	T	30	71(43.8)	91(56.2)	33(32.4)	69(67.6)	104(39.4)	160(60.6)
Aminoglycosides	Gentamicin	GM	10	19(11.7)	145(89.5)	23(22.5)	77(75.5)	42(15.9)	222(84.1)
	Streptomycin	S	300	89(54.9)	73(45.1)	49(48.0)	53(51.9)	23(8.7)	241(91.3)
	Kanamycin	K	30	33(20.4)	129(79.6)	20(19.6)	82(80.4)	53(20.1)	211(79.9)
	Neomycin	NE	10	57(35.2)	105(64.8)	31(30.4)	71(69.6)	88(33.3)	176(66.7)
	Cephalosporins	Ceftriaxone	CRO	30	89(54.9)	73(45.1)	21(20.6)	81(79.4)	110(41.7)
Carbapenems	Cefotaxime	CTX	30	14(8.6)	148(91.4)	15(14.7)	87(85.3)	29(11)	235(89)
	Ceftazidime	CAZ	10	35(21.6)	127(78.4)	17(16.7)	85(83.3)	52(19.7)	212(80.3)
	Imipenem	IMI	10	12(7.4)	150(92.6)	22(21.6)	80(78.4)	34(12.9)	230(87.1)
	Meropenem	MEM	10	24(14.8)	138(85.2)	9(8.8)	93(91.2)	33(12.5)	231(87.5)
	Ertapenem	ETP	10	8(4.9)	154(95.1)	10(9.8)	92(90.2)	18(6.8)	246(93.2)
Phenicols	Doripenem	DOR	10	13(8)	149(92)	5(4.9)	97(95.1)	18(6.8)	246(93.2)
	Chloramphenicol	C	30	35(21.6)	127(78.4)	25(24.5)	77(75.5)	60(22.7)	204(77.3)
Nitrofurans	Nitrofurantoin	NI	300	65(40.1)	97(59.9)	15(14.7)	87(85.3)	80(30.3)	184(69.7)

Table 4: Multiple antibiotic-resistant phenotypes (MARPs) pattern of *E. coli* isolates from the formal and informal meat sector

Pattern number	Number of Antibiotics	Pattern	Meat sector		MARI
			Formal	Informal	
1	3	T-TS-CIP	3	2	0.2
2	3	A-AMP-CIP	5	3	0.2
3	3	TS-AMP-A	1	0	0.2
4	3	GM-T-C	5	15	0.2
5	4	GM-AMP-A-TS	2	3	0.2
6	4	S-AMP-NI-CAZ	1	0	0.2
7	4	CAZ-CTX-CRO-TS	3	5	0.2
8	5	ETP-MEM-IM-DOR-CRO	2	6	0.3
9	5	TS-AMP-A-C-CAZ	0	3	0.3
10	6	MEM-CAZ-DOR-NI-T-C	1	2	0.3
11	6	K-AMP-GM-A-T-NE	2	0	0.3

12	6	MEM-NI-S-T-A-TS	2	2	0.3
13	7	A-AMP-TS-GM-IMI-NI-C	0	4	0.4
14	7	K-GM-MEM-NE-T-A-NOR	8	3	0.4
15	8	ETP-C-IMI-T-A-AMP-CTX-S	4	5	0.4
16	8	AMP-T-TS-NI-GM-CRO-ETP-A	0	2	0.4
17	8	CRO-ETP-A-MEM-IMI-NI-AMP-T	0	1	0.4
18	9	T-NE-K-AMP-NOR-TS-S-ETP-CTX	2	3	0.5
19	10	NI-CIP-AMP-T-TS-C-S-GM-IMI-A	0	2	0.5
20	10	C-GM-TS-T-CRO-ETP-NOR-NI-A-AMP	1	1	0.5

Table 5: Percentage and distributions of antimicrobial resistance determinants among *E. coli* from formal and informal sector

Antimicrobial agent	Disc code	Antimicrobial resistance gene	Gene group and or general function	Formal (%)	Informal (%)	Total (%)
Gentamicin (n = 42)	GM	<i>aac(3)-IIa (aacC2)a</i>	Aminoglycoside resistance	9(21.4)	13(31)	22(52.4)
Kanamycin (n = 53)	K	<i>aph(3)-Ia (aphA1)a</i>	Aminoglycoside resistance	11(20.8)	8(15.1)	19(35.8)
		<i>aph(3)-IIa (aphA2)a</i>	Aminoglycoside resistance	20(37.7)	10(18.9)	30(56.6)
Neomycin (n = 88)	NE	<i>aph(3)-Ia (aphA1)a</i>	Aminoglycoside resistance	8(9.1)	13(14.8)	21(23.9)
		<i>aph(3)-IIa (aphA2)a</i>	Aminoglycoside resistance	20(22.7)	10(11.4)	30(34.1)
Streptomycin (n = 138)	S	<i>aadA</i>	Aminoglycoside resistance	56(40.6)	44(31.9)	100(72.5)
		<i>strA</i>	Aminoglycoside resistance	9(6.5)	13(9.4)	22(15.9)
Amoxicillin (n = 45)	A	<i>blaTEM</i>	Beta-lactam	2(4.4)	6(13.3)	8(17.8)
		<i>blaZ</i>	Beta-lactam	4(8.9)	1(2.2)	5(11.1)
Ampicillin (n = 20)	AMP	<i>ampC</i>	Beta-lactam - <i>AmpC</i>	4(20)	9(45)	13(65)
Chloramphenicol (n = 60)	C	<i>cat1</i>	Chloramphenicol resistance	1(1.7)	1(1.7)	2(3.3)
		<i>cat2</i>	Chloramphenicol resistance	1(1.7)	0(0)	1(1.7)
		<i>cmlA1</i>	Efflux pump	1(1.7)	1(1.7)	2(3.3)
Cotrimoxazole (n = 45)	TS	<i>sul1</i>	Sulphonamide resistance	10(22.2)	12(26.7)	22(48.9)
		<i>sul2</i>	Sulphonamide resistance	8(17.8)	3(6.7)	11(24.4)
Tetracycline (n = 104)	T	<i>tetA</i>	Tetracycline resistance	8(7.7)	16(15.4)	24(23.1)
		<i>tetB</i>	Tetracycline resistance	12(11.5)	25(24)	37(35.6)
		<i>tetC</i>	Tetracycline resistance	1(1)	7(6.7)	8(7.7)
		<i>tetD</i>	Tetracycline resistance	1(1)	13(12.5)	14(13.5)
		<i>tetM</i>	Tetracycline resistance	2(1.9)	9(8.7)	11(10.6)

Table 6: Genotypic resistance determinants profile of *E. coli* isolates from the formal and informal meat sector

Antimicrobial class	Number of pattern	Genotype resistance determinants pattern	Formal	Informal	Total
Aminoglycosides	2	<i>aac(3)-IIa (aacC2)^a+aph(3)-Ia (aphA1)^a</i>	1	1	2
	3	<i>aac(3)-IIa (aacC2)^a+aph(3)-Ia (aphA1)^a+aph(3)-IIa (aphA2)^a</i>	2	0	2
	2	<i>aadA+strA</i>	4	1	5
Beta-lactams	2	<i>blaTEM+blaZ</i>	0	1	1
	2	<i>blaZ+ampC</i>	0	1	1
Phenicol	2	<i>catI+catII</i>	1	0	1
	2	<i>catII+cmlA1</i>	1	0	1
Sulphonamides	2	<i>sul1+sul2</i>	2	6	8
Tetracyclines	2	<i>tetA+tetB</i>	1	4	5
	2	<i>tetA+tetC</i>	3	9	12
	2	<i>tetB+tetD</i>	0	1	1
	3	<i>tetA+tetB+tetD</i>	0	5	5
	3	<i>tetC+tetA+tetB</i>	2	4	6
	3	<i>tetA+tetB+tetM</i>	1	2	3
	3	<i>tetB+tetD+tetM</i>	2	0	2
	3	<i>tetD+tetC+tetA</i>	1	0	1
	4	<i>tetA+tetB+tetC+tetD</i>	1	4	5
	4	<i>tetA+tetB+tetC+tetM</i>	1	3	4
4	<i>tetB+tetC+tetD+tetM</i>	2	4	6	

^a Alternative nomenclatures are in parentheses

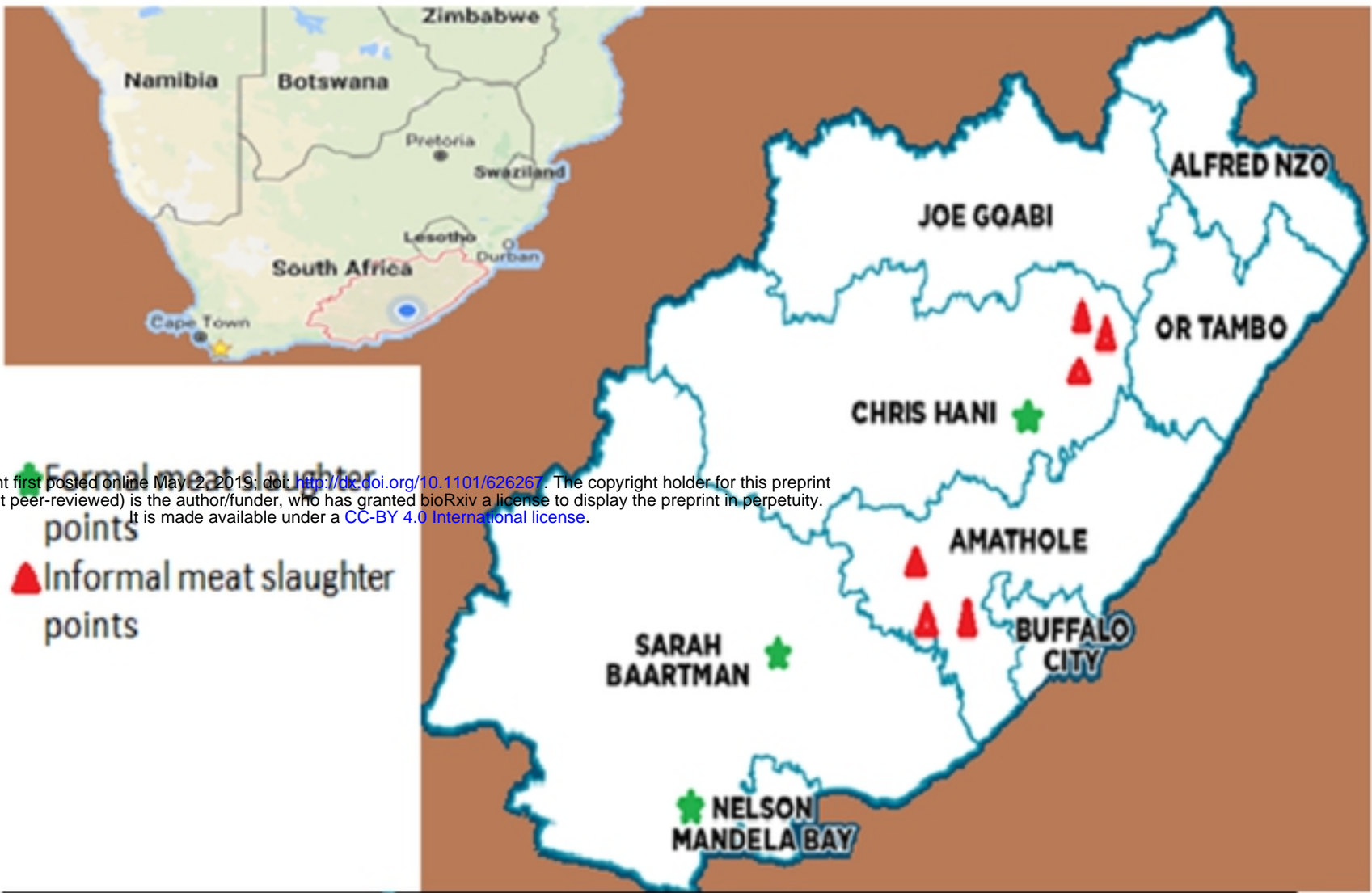
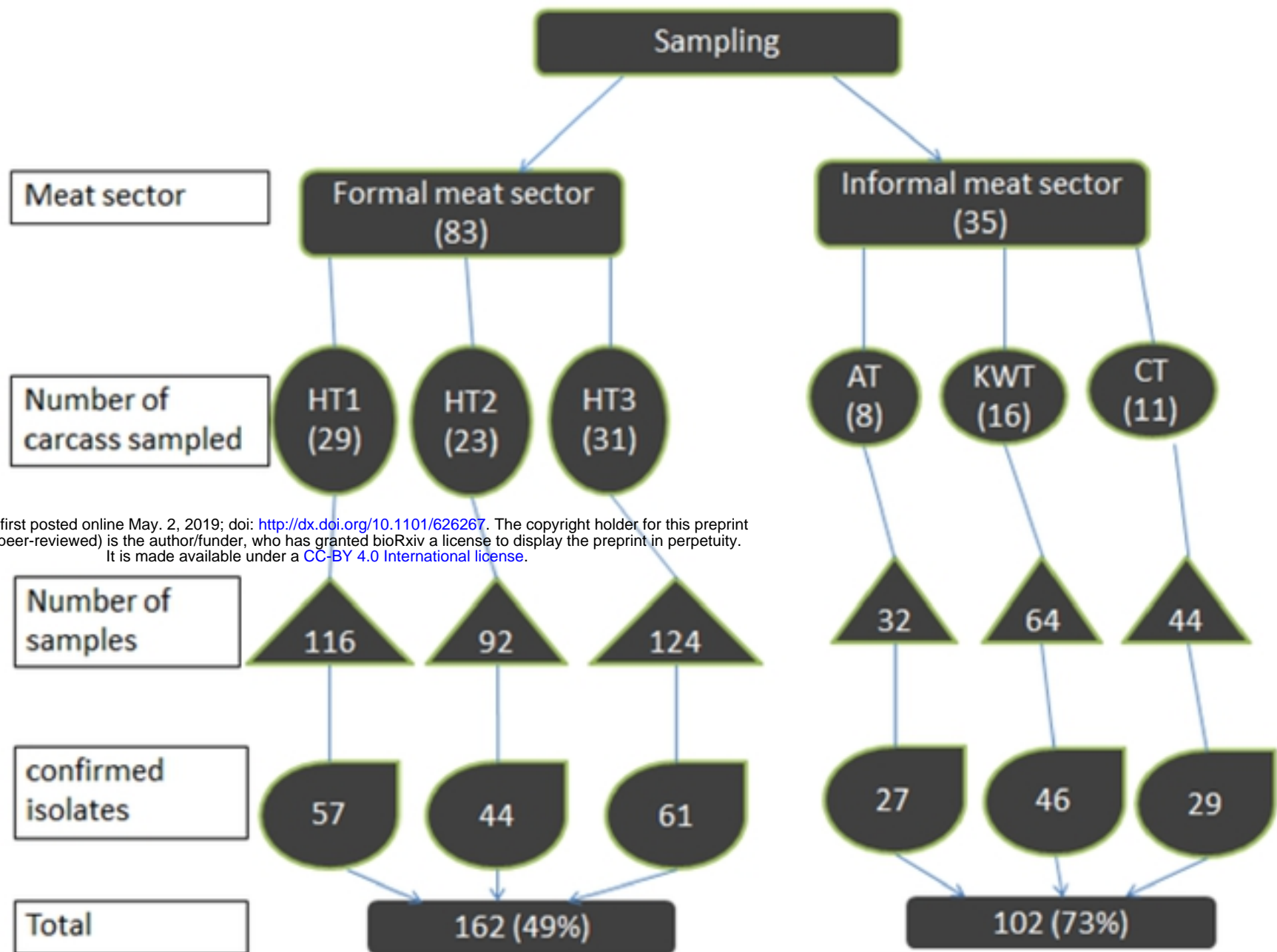


Figure 1: Map of the Eastern Cape Province showing sampling points



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Figure 2: Flow diagram detailing the sample collection from the formal and informal meat sector.