Antimicrobial drug resistance of *Escherichia coli* isolated from poultry abattoir workers at risk and broilers on antimicrobials

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ABSTRACT

Antimicrobial usage in food animals increases the prevalence of antimicrobial drug resistance among their enteric bacteria. It has been suggested that this resistance can in turn be transferred to people working with such animals, *e.g.* abattoir workers. Antimicrobial drug resistance was investigated for *Escherichia coli* from broilers raised on feed supplemented with antimicrobials, and the people who carry out evisceration, washing and packing of intestines in a high-throughput poultry abattoir in Gauteng, South Africa. Broiler carcasses were sampled from 6 farms, on each of which broilers are produced in a separate 'grow-out cycle'. Per farm, 100 caeca were randomly collected 5 minutes after slaughter and the contents of each were selectively cultured for *E. coli.* The minimum inhibitory concentration (MIC) of each isolate was determined for the following antimicrobials: doxycycline, trimethoprim, sulphamethoxazole, ampicillin, enrofloxacin, fosfomycin, ceftriaxone and nalidixic acid. The same was determined for the faeces of 29 abattoir workers and 28 persons used as controls. The majority of isolates from broilers were resistant, especially to antimicrobials that were used on the farms in the study. Overall median MICs and the number of resistant isolates from abattoir workers (packers plus eviscerators) tended to be higher than for the control group. However, no statistically significant differences were observed when the median MICs of antimicrobials used regularly in poultry and percentage resistance were compared, nor could an association between resistance among the enteric *E. coli* from packers and those from broilers be demonstrated.

Key words: abattoir workers, antimicrobial drug resistance transfer, broilers, *Escherichia coli,* MICs, oral antimicrobial therapy.

INTRODUCTION

Collibacillosis is known to contribute significantly to increased mortality and economic losses in the poultry industry[45]. As a result, antimicrobials, sometimes at sub-therapeutic concentrations, are often included in feed given to food animals to prevent disease, reduce mortality and morbidity, enhance feed conversion efficiency and improve growth rates[2,3,10,13,15,19,22,29,39,46]. However, in many countries of the world, legislation prohibiting the use of especially performance enhancers, but also certain therapeutics is applied to an increasing number of antimicrobials[2,4,24,40,41,45]. The reasons given are:

- there is a possibility of resistant bacterial strains, especially zoonotic bacteria, from food producing animals infecting humans[2,6,7,13,19, 22,25,37,43,44,47],
- there is potential for antimicrobial drug resistant bacteria of animals transferring resistance encoding genetic material to bacteria that are pathogenic in humans[2,5,13,19,25,34,36,40,41,43];
- when antimicrobials are used in an individual, they affect not only the micro organisms in the individual being treated, but also other people or animals that are in direct or indirect contact with that individual[1,17]. This is usually *via* the contamination of food, drinking water, transport vehicles or the application of manure to crops. This phenomenon has led to antimicrobials being designated as 'societal drugs'[1];
- after animal handlers have picked up resistant bacteria, they could pass them on to the human population at large[28]; and
- there is potential for antimicrobial usage in animals to induce cross-resistance to antimicrobials used in human medicine[3,4,12,43].

When studying resistance levels of bacteria from persons involved in animal handling, such as abattoir workers, enteric *E. coli* is considered to be the organism of choice as a model[28]. This is because *E. coli* strains efficiently exchange genetic material not only with each other, but also with other enteric pathogens such as *Salmonella, Yersinia* and *Vibrio* species[32]. Furthermore, studies with *E. coli* are of particular relevance because this species, which is a commensal of the intestines of both humans and animals, is a useful indicator of the antimicrobial resistance in bacteria in the community[32]. Frediani-Wolf[16] cited a study in the Netherlands that showed that farmers who work with turkeys fed antimicrobials as performance enhancers are likely to carry a higher level of resistant *E. coli* than their compatriots who worked with pigs that had not been fed with these products. A recently concluded study in South Africa on poultry abattoir workers, who carry out evisceration of broilers fed feed with antimicrobials, showed that people associated with poultry abattoirs harbour bacteria with higher levels of resistance than people not associated with poultry abattoirs[31].

South Africa is one of the few countries in the world with a large intensively-reared poultry population in which performance enhancing as well as therapeutic antimicrobials are extensively used. However, information on antibacterial resistance in bacteria of poultry is scanty in this country, with isolated studies that rapidly become outdated owing to changing antimicrobial treatment practices. In these studies[19,23] retail carcasses were sampled, and it was found that 98–100 % of isolated *Salmonella* were resistant to tetracyclines. Of the staphylococci tested, resistance to both tetracycline and oxacillin was 39 % for retail chicken and 70 % for abattoir poultry isolates. Resistance among the Enterobacteriaceae isolates to

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both tetracycline and streptomycin was 34 % for retail isolates, and 60 % for abattoir poultry isolates. It is noteworthy that a large proportion of the bacterial flora from fresh poultry in these studies exhibited multiple antimicrobial drug resistance (MAR), *i.e.* many isolates were resistant to 3 or more antimicrobials. Even the recently concluded studies by both the South African National Veterinary Surveillance and Monitoring Programme for Resistance to antimicrobial Drugs[37] and Oguttu[31], confirm that resistance is high among poultry isolates. In spite of these reports and the fact that the veterinary profession in South Africa is aware of the emergence of antimicrobial resistance (based on laboratory data) and the need to investigate it, according to Nel and others[28,31], surveillance programmes for antimicrobial resistance are only in their infancy, with a veterinary surveillance programme only recently initiated[37]. In view of this, there is a need for studies on antimicrobial drug resistance in this country to supplement the budding veterinary surveillance programme.

In South Africa *mala* (intestines) from chickens fed performance enhancers and possibly carrying microorganisms that are resistant to antimicrobials are processed (cleaned and packed) by abattoir workers before being sold to consumers[31]. This implies that abattoir workers are exposed to potentially resistant microorganisms during their work, and could therefore be at risk of developing resistance among their enteric flora[31,35]. It is remarkable that this potential risk has not been extensively investigated.

Since the potential risk for resistance transfer from broilers to abattoir workers has not been investigated extensively in South Africa, the primary objective of this study was to investigate whether the prevalence of resistant enteric *E. coli* is higher in abattoir workers who eviscerate, wash and pack intestines from chickens fed feed medicated with antimicrobials than in people who do not work in poultry abattoirs. To do this the level of antimicrobial drug resistance of isolates from the abattoir workers whose work includes *mala* washing and packing was compared with that of people not associated with the abattoir.

Given that there is a need for data on antimicrobial drug resistance to supplement the budding veterinary surveillance programme in South Africa, this study also sought to elucidate the occurrence of antimicrobial resistance in commensal *E. coli* isolated from broilers on a group of farms in the Gauteng area where antimicrobials are included in their feed or drinking water.

MATERIALS AND METHODS

Broiler specimen collection

Broiler carcasses were sampled from 6 farms from different 'grow-out cycles'. One hundred ($n = 100$) caeca were randomly collected per farm from slaughtered broilers approximately 5 minutes after slaughter at a high throughput poultry abattoir in South Africa. Samples were taken at a point on the slaughter line after the 1st inspection point, where carcasses with defects are identified and removed either to be condemned or to be cut up as portions. This was to ensure that the chickens sampled had been healthy before slaughter and therefore fit for human consumption.

The specimens were harvested by aseptically incising the caeca off the rest of the gastrointestinal tract. They were then tied off at the open end so as to prevent contamination. The caeca were then placed in separate sterile plastic bags and conveyed in an insulated polystyrene container with frozen ice packs to the laboratory for processing within 3 hours of harvesting. Out of a total of 100 caeca sampled from each farm (with the exception of the first 100 caecum samples collected in the pilot project that were all plated out to isolate the relevant bacteria), 25 caeca on some farms and 30 caeca on others (depending on the time available to complete the plating out of the specimen), were randomly selected from each farm, and used to culture of *E. coli*. However, all 100 caeca were used for the selective culture of salmonellae that was also investigated in the same study.

Human specimen collection

Only abattoir workers located in the evisceration and intestine (*mala*) packing areas of the abattoir were included in the study group. Furthermore, only people in the designated areas who had not been on any form of antimicrobial therapy for at least 3 months prior to sampling were requested to provide a faecal sample. Out of a possible 44 people, 29 volunteered and qualified to participate in the study.

Volunteers consisting of students and workers at the Faculty of Veterinary Science, University of Pretoria constituted the control group. Like the experimental group (abattoir workers), selection was purposive, and only people who had not been on antimicrobials for at least 3 months prior to sampling were requested to provide a sample. In addition, people identified and selected to act as the control group were required not to have been in contact with or handled poultry or animal feed during the period of sampling or for at least 3 months prior to

sampling. Twenty-eight people agreed to act as anonymous volunteers and completed informed consent forms before providing a sample. It was not possible to exclude persons who had eaten poultry products.

Each volunteer was given a bottle with a spoon to collect the morning stool, by scooping off either the first or last faeces from the anal area. No faecal sample for submission was to be picked from the ground or toilet. The sample bottles with stool were brought to the company clinic (in a cooler box with ice packs) located on the premises of the abattoir as the volunteers reported for work in the morning. The samples were then transported to the bacteriology laboratory of the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, to isolate *E. coli* and salmonellae.

Culture and identification of E. coli

E. coli and salmonellae were cultured and identified using standard methods[31]. In brief, lactose fermenting colonies on MacConkey agar (Oxoid products, Basingstoke, UK) were purified by streaking onto Columbia agar (Oxoid products, Basingstoke, UK) containing 5 % citrated horse blood and identified as *E. coli* if they were Gram-negative bacilli, motile, fermentative, oxidase-negative, catalase- and indole-positive, citrate negative and malonate negative. After a series of enrichments in peptone water (Oxoid products, Basingstoke, UK), and selection in Rappaport broth (Difco Laboratories, Detroit, USA) and XLD agar (Difco Laboratories, Detroit, USA) respectively, black colonies surrounded by pink zones were subjected to the API20S test (BioMerieux, France) to determine whether they were *Salmonella enterica.*

Selection of antimicrobials for testing

Antimicrobials selected for minimum inhibitory concentration (MIC) testing included ceftriaxone, a 3rd generation cephalosporin and nalidixic acid, neither of which are registered for use in poultry, but used extensively in human medicine in South Africa. Furthermore, nalidixic acid was included to detect low-level resistance to the fluoroquinolones. All the members of the tetracyclines and sulphonamides respectively have the same mode of action and can therefore be represented by 1 member of the group *i.e.* doxycycline in the case of the tetracyclines and sulphamethoxazole for the sulphonamides. Although trimethoprim is usually used in combination with a sulphonamide, it was tested separately to ascertain the origin of resistance to this antimicrobial. The ampicillin and the

slightly more lipid-soluble amoxycillin are analogues and thus the more stable ampicillin was used in AST as a representative of the beta-lactam antimicrobials. Tetracyclines, amoxycillin, potentiated sulphonamides (sulphamethoxazole and trimethoprim), enrofloxacin and fosfomycin had been used in the flocks under study for the duration of the sampling period.

Antimicrobial susceptibility testing

One *E. coli* isolate was selected from each sample and subjected to the MIC micro-broth dilution test as prescribed by the Clinical and Laboratory Standards Institute USA (CLSI)[26], to determine the susceptibility of the isolates to the above-mentioned antimicrobials. As recommended [21,26,31], pure antimicrobial powders were used (Sigma-Aldrich, USA) and the antimicrobial potency of each product (μg or international units (IU)/mg) was calculated using proportional molecular weights and percentage purity of each antimicrobial. The diluents chosen were those recommended by the CLSI or by the product manufacturers. Two-fold dilutions of each antimicrobial were made in sterile 96-well U-bottomed plates (Sterilab, South Africa). The minimum antimicrobial drug concentration where there was no visible growth of bacteria was recorded and the isolate determined as either susceptible or resistant based on microbiological cut-off concentrations used by the CLSI[26]. In the case of drugs for which CLSI does not provide the cut-off point, figures from the Swedish and Spanish surveillance programmes were used[38,42]. The reference points used were: ampicillin 1 mg/ , ceftriaxone, 1 mg/ , doxycycline 8 mg/ , enrofloxacin 0.25 mg/ , nalidixic acid 8 mg/ , fosfomycin 16 mg/ , sulphamethoxazole 256 mg/ and trimethoprim 8 mg/ .

Data analysis

Recorded data were analysed by the statistical package Stata 8.2 (StataCorp, College station, TX, USA). A *P*-value < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Isolates

Not all samples yielded an isolate and in such cases no attempt was made to re-culture them. The number of *E. coli* cultured is summarised in Table 1, and included 168 from broilers, 28 from abattoir workers and 26 from the control group. As the farms used in the study had an intensive salmonella eradication programme, no salmonellae were isolated.

Table 1: **Number of *Escherichia coli* selected for MIC testing from the different populations sampled.**

Source	Number of isolates
Broilers	168
Abattoir workers	28
Control group	26

This means that these farms were able to control salmonellae, but this is not true of the national flock, where salmonellae are routinely isolated[37].

The majority of the *E. coli* isolates from broilers had MIC values that were considered to indicate resistance to doxycycline (98.2 %), sulphamethoxazole (78.7 %), ampicillin (75 %), enrofloxacin (75.6 %) fosfomycin (98.2 %) and nalidixic acid (90.5 %). All with the exception of nalidixic acid were administered orally to the flocks studied either as feed additives or for prophylactic purposes. These levels of resistance were anticipated given that use of antimicrobials as feed additives in animals is known to lead to development of resistance among enteric organisms from such animals. These findings are consistent with previous and recent studies that reported a high level of resistance among isolates from broilers in South Africa[19,23,37].

A single mutation in the quinolone resistance-determining region (QRDR) of the topoisomerase gene *gyrA* (commonly at positions 83 and 87) usually leads to resistance against nalidixic acid, a non-fluorinated narrow-spectrum quinolone and to decreased fluoroquinolone susceptibility among Gram-negative bacteria, *e.g. Salmonella* and *E. coli,* whereas 2 or more mutations in *gyrA* or *parC* lead to high resistance to the fluoroquino-lones[14,33,34]. It is therefore expected that resistance to nalidixic acid would be higher than that of enrofloxacin. The high level of resistance to enrofloxacin is not considered unusual in South Africa. In fact a study by SANVAD[37] confirms that resistance to enrofloxacin among healthy broiler isolates in South Africa is high (65.2 %). This is possibly not only due to the use of enrofloxacin but also as a result of cross-resistance when other fluoro-quinolones such as norfloxacin are used.

The prevalence of resistance to ceftriaxone (39.3 %), although low compared with what was observed for other antimicrobials among broiler isolates, was unexpected. However, cephalosporin resistance among avian *E. coli* isolates has been reported previously[44]. It is postulated that exposure of *E. coli* to low levels of tetracycline induces an expression of genetic loci that regulates susceptibility to

cephalosporins, penicillin, chloramphenicol, tetracycline, nalidixic acid and fluoroquinolones[27]. Since the flocks sampled had been on tetracycline at the time of sampling, this could account for the level of resistance observed to ceftriaxone (a cephalosporin) even though the isolates tested had not been exposed to these antimicrobials at the time. Furthermore, it is known that mutations in *marR* of the *marRAB* operon lead to multiple-antibiotic resistance among *E. coli* isolates[14]. The primary cause of resistance in a large number of Gram-negative bacilli like *E. coli* is the ability to generate Extended-Spectrum Beta-Lactamase (ESBL) enzymes that can inactivate the penicillin and cephalosporin class antibiotics. In addition, this type of resistance is known to manifest rapidly[27]. It is therefore also possible that these *E. coli* isolates exhibit ESBL. This resistance to ceftriaxone could also be attributed to cross resistance with amoxycillin, a β-lactam to which the isolates were exposed.

Since broilers only live for 35–42 days and the farms sampled, according to the questionnaire completed, practise an all-in all-out system of rearing, with poultry houses thoroughly cleaned, washed and disinfected before new batches of broilers are brought into the poultry houses, the high level of resistance observed here demonstrates the ability of bacteria to develop resistance quickly or the ability of a few resistant bacteria that survive to quickly re-populate the flock when exposed to antimicrobial selection pressure. A study of *Campylobacter* in the USA showed that chickens naturally colonised with fluoroquinolone-susceptible strains began excreting resistant strains after 2 days of doses of enrofloxacin, a drug commonly used for prophylaxis and treatment in the poultry industry[20]. In another study cited by Gouws and Brözel[19], it was demonstrated that all Enterobacteriaceae from chickens fed with tetracycline-treated feed developed resistance within 36 to 48 hours. Within 3 months, resistance to tetracycline observed in the cited study was accompanied by resistance to ampicillin, carbenicillin and sulphonamides. This multiple resistance was subsequently accompanied by an increased ability of these strains to transfer tetracycline resistance.

With the exception of trimethoprim, it is clear that the prevalence of resistance among the broilers is much higher than that observed among the isolates from the 2 human populations that were sampled (Fig. 1). This could be attributed to the fact that the conditions of antimicrobial usage in farm animals favour the
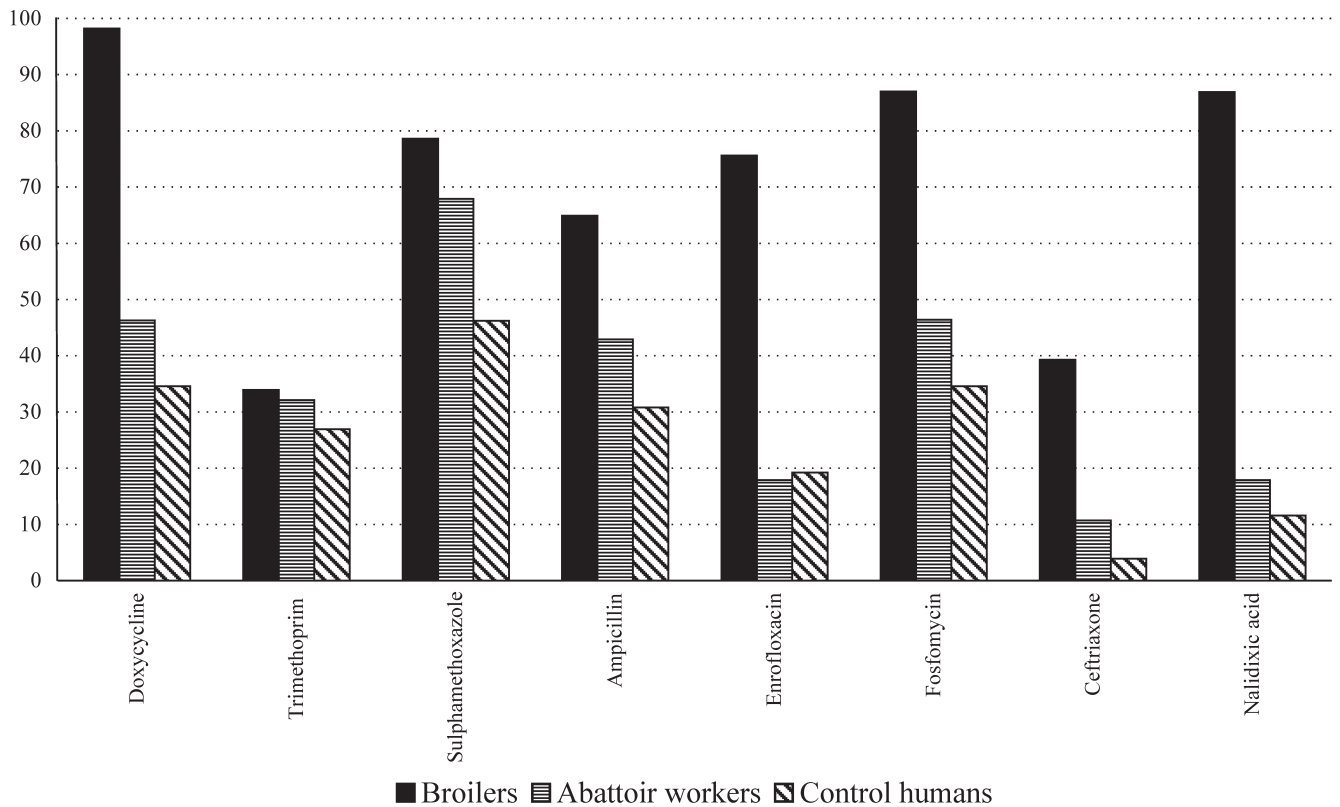
Fig. 1: **Percentage resistance of *Escherichia coli* from broilers** ($n = 168$)**, packers** ($n = 28$) **and human controls** ($n = 26$) **to antimicrobial drugs tested in this study.**

development of resistance in comparison to the situation in humans[15].

Figure 1 also shows that the level of resistance tended to be higher among abattoir workers (with the exception of enrofloxacin) than in the control group. This supports the suggestion that people working with animals fed feed with antimicrobial additives tend to have a higher level of resistance to such antimicrobials than those who do not[28,40]. However, statistical analysis using the Wilcoxin rank-sum test showed no significant differences between abattoir workers and the control group for all the antimicrobials ($P > 0.05$). Notwithstanding these findings, with the exception of ceftriaxone, to which 3.9 % *E. coli* isolates from the control group had MICs considered resistant, resistance among *E. coli* isolates from the 2 human populations tested could still be described as being very high or similar to what it was in Europe before the use of feed growth enhancers was abolished[8,9,43]. However, the levels of resistance observed here are much lower than in countries where antimicrobials are easily accessible and are available over the counter. For example, in Nigeria a prevalence of up to 90 % resistance to tetracycline among human isolates has been recorded[30]. This could be attributed to the less stringent regulatory mechanisms in such countries compared with South Africa, where antimicrobials are not easily accessible over the counter for use in human medicine.

The difference in the number of *E. coli* isolates with MICs above the cut-off point for fosfomycin between the workers (46.6 %) and the control group (34.6 %) was not significant ($P = 0.418$) (Fig. 1). In South Africa, fosfomycin trometamol (Urizone, Adcock Ingram (Pty) Ltd, South Africa) is only occasionally used for the treatment of cystitis in humans, so the finding was unusual. It was anticipated that abattoir workers by virtue of their closeness to poultry isolates with a high level of resistance, would carry a higher level of resistance due to resistance transfer than the control group. However, resistance in this phosphoenol pyruvate analogue can be conferred by the transmissible *phosA* gene. In a study in England where fosfomycin is not used it was found that this gene was present in *E. coli* isolates from urinary tract infections of humans as well as bacteria isolated from animal products from Spain, a country that uses this antimicrobial[18]. It was therefore proposed that fosfomycin resistance originates from eating animal products. The same may be true for this study given that it was not possible to exclude persons who were eating poultry products and that fosfomycin is widely used as a feed additive in South African poultry flocks.

The numbers of *E. coli* isolates from the abattoir workers with median MIC values above the cut-off point were lower than was observed for poultry isolates for the following antimicrobials: doxycycline

($P < 0.001$), enrofloxacin ($P < 0.001$), fosfomycin ($P < 0.001$), ceftriaxone ($P = 0.003$) and nalidixic acid ($P < 0.001$). For trimethoprim ($P = 1.00$), sulphamethoxazole ($P = 0.228$) and ampicillin ($P = 0.350$), no significant differences were observed when the median MIC values were compared. The former group consisted of antimicrobials extensively used in poultry and for which the antimicrobial selection pressure would be greater in broilers than in humans.

As expected, *E. coli* isolates from people not associated with the abattoir (control group), likewise had lower median MICs than broiler isolates. Significant differences were observed for doxycycline ($P < 0.001$), sulphamethoxazole ($P < 0.001$), ampicillin ($P = 0.002$), enrofloxacin ($P < 0.001$), fosfomycin ($P < 0.001$), ceftriaxone ($P < 0.001$), and nalidixic acid ($P < 0.001$). The exception was trimethoprim ($P = 0.654$) for which a level of percentage resistance close to that observed among *E. coli* isolates from poultry was recorded. This is attributed to the fact that selection pressure for resistance to the antimicrobials tested (with the exception of trimethoprim) is highest among poultry isolates.

Since the packers have a higher exposure to enteric bacteria from poultry, it was decided to compare the median MICs of the *E. coli* originating from the packers to that of the eviscerators. Even though the packers did have slightly higher
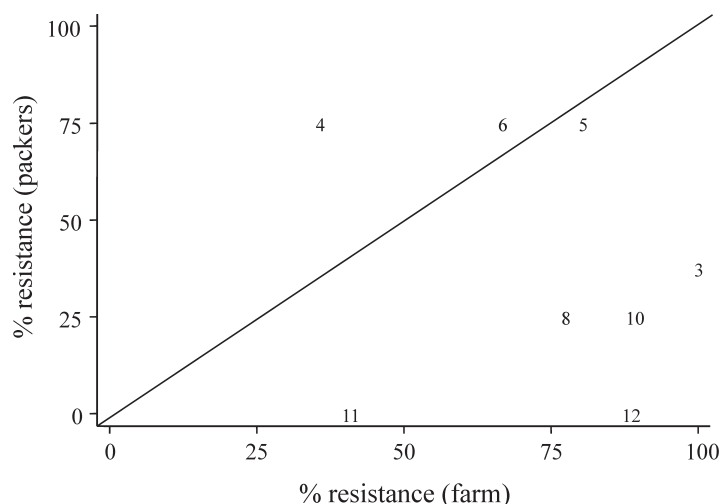
Fig. 2: **Scatter plot for percentage resistant isolates from packers and broilers for each antimicrobial drug. 3 = Doxycycline; 4 = trimethoprim; 5 = sulphamethoxazole; 6 = ampicillin; 8 = enrofloxacin; 10 = fosfomycin; 11 = ceftriaxone and 12 = nalidixic acid.**

median MICs than the eviscerators, statistically significant higher values were only detected for trimethoprim ($P = 0.002$), ampicillin ($P = 0.041$) and lower values in the case of nalidixic acid ($P = 0.022$). Significant differences between the packers and control groups were also observed for trimethoprim ($P = 0.012$) and ampicillin ($P = 0.036$). However, higher median MICs were recorded for the *E. coli* isolates from the control group compared to the packers for doxycycline, fosfomycin and the fluoroquinolone early resistance indicator, nalidixic acid. As the last 3 classes are more commonly used in poultry, the results were contrary to what was expected. It appears that the packers are not more likely to carry more resistant genes than the eviscerators.

The rank correlation coefficient was determined for isolates from the packers and the broilers. The existence of significant levels of resistance transfer between the 2 populations could have been suggested if most of the drugs (represented by numbers in Fig. 2) lay close to the diagonal line through the graph. However, as demonstrated by the scatter plot percentage on the graph in Fig. 2, no correlation (Spearman's $r = 0.21$, $P = 0.62$) was observed between *E. coli* isolates from 2 populations. This implies that resistance transfer from animals to humans was not occurring at statistically significant levels in this poultry abattoir.

CONCLUSIONS

Broilers sampled carried an exceptionally high level of resistance to antimicrobials frequently used in poultry, namely tetracyclines, fluoroquinolones, penicillins, fosfomycin and sulphonamides, which might reflect what could be happening in the Gram-negative enteric population of bacteria of the national broiler

flocks. In view of this, it is recommended that the South African National Veterinary Surveillance and Monitoring programme for resistance to Antimicrobial Drugs (SANVAD) receive the full support of government, veterinarians and the farming community to be able to establish trends in antimicrobial drug resistance in this country. The importance of this is appreciated when consideration is given to the fact that emergence of antimicrobial resistance phenotypes among food-borne bacteria[11,44] implies the likelihood of failure of empiric treatment of food-associated diseases[11].

In view of this, it is recommended that the poultry industry and in particular the farms in this study adopt a prudent antimicrobial usage policy or even consider moving to a high health status with minimum antimicrobial usage. The latter programme has been successful in some European countries where there was no marked loss in production[2,4,19,46,47]. Furthermore, it is recommended that all antimicrobials for use in poultry become prescription drugs and that poultry farms base any treatments on antimicrobial susceptibility tests.

While these results confirmed that abattoir workers generally carried higher levels of resistance, statistical analysis did not show significant differences in the level of resistance between the 2 human populations (abattoir workers and control group) studied for any of the antimicrobials commonly used for poultry health and production. This implies that high levels of antimicrobial resistance are as a result of both animal and human antimicrobial drug usage and that cross-resistance could be a common event.

The unexpectedly high levels of fosfomycin resistance in humans is of concern because this product is being used almost

exclusively in poultry. However, since people in South Africa eat large quantities of poultry meat and eggs, the possibility that these products are sources of resistant bacteria and/or antibiotic residues for the human population should be further investigated.

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