Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga, South Africa

Charlotte Ropafadzo Mupfunya1 | Daniel Nenene Qekwana2 | Vinny Naidoo2

Abstract
Surveillance of antimicrobial use and antimicrobial resistance is a critical component of the “Global Action Plan” to tackle antimicrobial resistance. However, there is a paucity of such surveillance in communal farming areas in South Africa. This study investigated knowledge and antimicrobial use practices by cattle farmers and antimicrobial resistance levels of indicator bacteria in cattle in a rural communal farming area in South Africa. Seventy (70) farmers were interviewed at five cattle inspection sites using structured questionnaires. Rectal swabs were collected from apparently healthy cattle (n = 100) for culture of Escherichia coli and Enterococcus species, and antimicrobial susceptibility testing using broth microdilution. The farmers indicated predominantly using tetracyclines. Although 42% of the farmers indicated hearing about antimicrobial resistance, none of them clearly understood what it involves. Seventy-nine (79) E. coli and 71 Enterococcus species were isolated with E. faecium being the dominant species among the Enterococcus isolates. E. coli isolates were resistant to colistin (16%; 13/79), chlortetracycline (8%; 6/79) and amoxicillin (8%; 6/79). Enterococcus isolates were resistant to enrofloxacin (55%; 39/71) and amoxycillin (3%; 2/71). We observed knowledge gaps in prudent antimicrobial use practices and antimicrobial resistance among the farmers. Farmer tailored education programmes on primary animal health care and prudent antimicrobial use practices must be developed and implemented to improve antimicrobial stewardship among farmers with limited veterinary supervision. The level of colistin resistance detected among E. coli isolates from rural communal cattle in this study was unexpected and warrants further molecular investigation to check if the resistance is plasmid mediated.

KEYWORDS
antimicrobial resistance, antimicrobial use, communal farming, Enterococcus, Escherichia coli, surveillance

1 | INTRODUCTION

Livestock rearing in communal farming areas in South Africa is an important means of diversifying livelihoods and alleviating poverty (Meissner, Scholtz, & Palmer, 2013). In communal farming areas, livestock are kept on separate households at night but graze together on communal pastures. The communal farming system has several challenges including high disease occurrence and poor veterinary
extension services (Mutibvu, Maburutse, Mbiriri, & Kashangura, 2012). As a countermeasure, the South African government through the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 36 of 1947 permits for over-the-counter availability of antimicrobials such as tetracyclines, sulphonamides, cloxacinil intramammary, fosfomycin, tylosin and kitsamycin, to allow for timely treatment of easily recognisable endemic diseases (Henton, Eagar, Swan, & van Vuuren, 2011; Naidoo, 2009).

Unfortunately, availing antimicrobials for use by farmers without much restriction increases the likelihood of indiscriminate antimicrobial use, a recognised key driver of antimicrobial resistance development (Aarestrup, 2015). Antimicrobial resistance is on the increase and is currently a critical threat to human and animal health due to the associated increased health care expenses, treatment failure and high mortalities (Roca et al., 2015).

Surveillance is one of the five strategic objectives outlined in the World Health Organisation (WHO) “Global Action Plan” for tackling antimicrobial resistance (WHO, 2015a). It allows for timely detection of emerging resistance and drivers of resistance, and guides intervention strategies (Aarestrup, 2015). *Escherichia coli* and enterococci have been selected as indicator bacteria for monitoring resistance because they easily develop resistance (Caprioli, Busani, Martel, & Helmuth, 2000; van Vuuren, Picard, & Greyling, 2007) and also serve as reservoirs of antimicrobial resistance genes which are transferrable to pathogenic bacteria (Caprioli et al., 2000; Varga et al., 2008). In addition, these microorganisms are present in both healthy and sick individuals and therefore provide a better picture of the antimicrobial resistance patterns in a population compared to pathogenic bacteria (Caprioli et al., 2000).

Livestock farmers in South Africa have access to certain antimicrobials without veterinary supervision. However, there is paucity of information on their knowledge of antimicrobials and antimicrobial use practices, more so for communal farmers. In addition, data on antimicrobial resistance patterns of indicator bacteria in communal cattle are also limited. Therefore, this study investigated knowledge on antimicrobial use practices and antimicrobial resistance levels of indicator bacteria in cattle in a communal farming community in South Africa. Information generated from this study can be used to guide policy on the availability of over the counter veterinary antimicrobials in South Africa to contribute towards prudent antimicrobial use.

2 | MATERIALS AND METHODS

2.1 | The study area

The study was conducted in the Mnisi community, a rural communal farming community in the Bushbuckridge Municipality area, Mpumalanga Province South Africa. The community spans over 29,500 hectares of land and is subdivided into four wards which consist of an estimated 8,500 homesteads with over 40,000 individuals. The municipality is characterised by high unemployment and low household incomes. Animal husbandry is the major agricultural activity in the area with cattle farming dominating (Berrian et al., 2016). The livestock herds share the same grazing areas (i.e. communal pastures) during the day but are penned at the owners’ households during the night. The area is semi-arid hence most livestock are prone to water and pasture shortages. Tick-borne diseases such as corridor disease, anaplasmosis, heartwater and babesiosis commonly occur in the area (Choopa, 2016).

Over two thirds of the community’s borders are shared with game parks. Consequently, there is spill over of foot and mouth disease (FMD) and tick-borne diseases from wildlife to livestock and vice versa. The cattle are thus required by law to be presented weekly for tick control using acaricides, and for inspection for foot and mouth disease lesions at designated communal inspection sites (Berrian et al., 2016). There were 21 inspection sites distributed across the four wards.

Each ward is serviced by one Animal health technician (AHT) (a veterinary paraprofessional) who serves as the primary veterinary personnel contact for the communal farmers. The farmers can buy veterinary drugs from the local animal clinic or an agricultural cooperative store in a neighbouring town which sells agricultural supplies including over-the-counter antimicrobials such as tetracyclines and sulphonamides. For technical reasons, sample collection was limited to all the five inspection sites of one ward.

2.2 | Study population

The study was conducted from January to February 2018. Invitation to participate in the study was extended by word of mouth to farmers at the five cattle inspection sites. All farmers willing to participate were interviewed and their cattle selected for sample collection. In total, 70 farmers were included in the study.

2.3 | Questionnaire survey

Face-to-face interviews based on a standardised questionnaire, translated to the local language, were conducted with participating farmers with the assistance of a local environmental health monitor (EHM). The farmers were interviewed using Shangaan, which is the local language and their responses to open-ended questions were translated by the EHM. The translated questionnaire was pretested using a mock interview prior to the survey to ensure that the meaning of the questions was not lost during translation.

2.4 | Farmer questionnaire

The questionnaire included both open and close-ended questions for demographic data, livestock species kept and duration of cattle rearing, cattle herd size, antimicrobials used on the farm and where they source them, disposal of expired antimicrobials and knowledge on antimicrobial resistance and importance of observing antimicrobial withdrawal periods.
2.5 | Animal health technician questionnaire and veterinarian interviews

A questionnaire (supporting information) with a different set of questions from the farmers’ questionnaire was administered to two AHTs working in the area to obtain information on antimicrobials commonly used and factors that may lead to resistance spread in their community. Three veterinarians who had worked at the local animal clinic were asked to name the veterinary antimicrobials they had commonly prescribed for use in the area.

2.6 | Sample collection

One hundred \((n = 100)\) cattle belonging to the interviewed farmers were randomly selected for this study. These cattle were apparently healthy (i.e., no visible clinical signs) and at least 6 months old. The number of cattle to be sampled at each dip tank was determined using a proportional stratified sampling approach to ensure a good geographical spread of the sample. One to three cattle were randomly selected per farmer as the cattle passed through the race. A single rectal swab sample was obtained from each selected cattle using sterile dry culture swabs. On each day, samples were transported to the laboratory on ice within 3 hr of collection and then stored at \(-80^\circ\text{C}\) for 1–3 weeks while awaiting processing.

2.7 | Bacterial culture and isolation

The faecal swabs were inoculated onto Columbia agar with 5% horse blood and then onto MacConkey agar without crystal violet (Thermo Fischer Scientific, South Africa). The plates were then streaked out using the quadrant streaking method. The blood agar plates were incubated with 5% carbon dioxide and both plates were incubated at 37°C for 24 hr. Suspect \(E.\ coli\) and enterococci were subcultured on the blood agar and MacConkey agar without crystal violet to obtain pure cultures. Gram-positive, catalase (Davies Diagnostics, South Africa) negative cocci occurring singly or in pairs and producing pinpoint red colonies on MacConkey (i.e., lactose fermenters) and positive for aesculin (Thermo Fischer Scientific, South Africa) hydrolysis were subjected to Streptococcal grouping using a commercial test kit, Streptex kit (Thermo Fischer Scientific, South Africa). Colonies falling in Lancefield group D were subjected to further sugar tests (Thermo Fischer Scientific, South Africa) to allow differentiation of some of the Enterococcus species according to the criteria described by Quinn, Carter, Markey, and Carter (1994). Some enterococci could not be identified to species level due to the limited array of sugars tested.

Gram-negative, catalase-positive and oxidase-negative, medium-sized rods producing large pink colonies on MacConkey agar were presumed to be \(Enterobacteriaceae\) if positive for indole (Merck, South Africa) production and then subjected to API 10S test (BioMerieux, South Africa) to identify \(E.\ coli\) isolates. One \(E.\ coli\) and/or one Enterococcus species if cultured were isolated from each animal sampled.

2.8 | Antimicrobial susceptibility testing

A micro-titre broth dilution method using water-soluble antimicrobial powders (Sigma Aldrich, Germany) was used to determine the susceptibility profile of each isolate following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2018a). \(E.\ coli\) isolates were subjected to antimicrobial susceptibility testing to the following agents; colistin sulphate, amoxicillin, enrofloxacin, chloramphenicol and gentamicin, while enterococci isolates were tested against amoxicillin, chloramphenicol, erythromycin, vancomycin and enrofloxacin (Sigma Aldrich, Germany). The tested antimicrobials fall under the group of antimicrobials recommended for routine antimicrobial surveillance testing studies for \(E.\ coli\) and enterococci (Caprioli et al., 2000; Franklin et al., 2001). Due to resource limitations, testing was limited to last resort antimicrobials (vancomycin and colistin), tetracycline commonly used by farmers in the study area and a few other antimicrobials. Water-soluble antimicrobial powders were used. The weight of each antimicrobial powder to be used was calculated based on the percentage purity of the powder and then dissolved in sterile water. The antimicrobial stock solutions were further diluted with the Mueller Hinton broth as necessary. Each of the antimicrobials was tested in duplicate in serial twofold dilutions from 0.25 \(\mu\text{g/ml}\) to 32\(\mu\text{g/ml}\). Clinical breakpoints were used to categorise isolates into susceptible, intermediate or resistant (Table 1) (CLSI, 2018b). \(E.\ coli\) ATCC #25922 and \(E.\ faecalis\) ATCC # 29,212 were used as controls. Isolates that were resistant to at least one antimicrobial were defined as resistant while those resistant to three or more antimicrobials were defined as multidrug resistant (Magiorakos et al., 2012).

| MIC Interpretive criteria (\(\mu\text{g/ml}\)) | \(E.\ coli\) | Enterococci |
|---------------------------------------------|-----------------|---------------|
| Susceptible                                | Intermediate    | Resistant     |
| ≤8                                         | ≤32             | ≥16           |
| ≤4                                         | ≤16             | ≥8            |
| ≤4                                         | ≤16             | ≥2            |
| ≤0.25                                      | 0.5–1           | ≥2            |
| ≤2                                         | ≥4              |               |
| Amoxicillin f                              |                 |               |
| Gentamicin                                 |                 |               |
| Tetracycline                               |                 |               |
| Enrofloxacin a                             |                 |               |
| Colistin b                                 |                 |               |
| Enterococci                                |                 |               |
| Amoxicillin f                              | ≤8              | ≥16           |
| Tetracycline                               | ≤4              | ≥16           |
| Erythromycin                               | ≤0.5            | 1–4           | ≥8 |
| Vancomycin                                 | ≤4              | 8–16          | ≥32 |
| Enrofloxacin a                             | ≤0.25           | 0.5–1         | ≥2 |

fBreakpoint for bovine respiratory disease was used.
bColistin breakpoint for Pseudomonas aeruginosa was used.
cAmpicillin breakpoint used.

TABLE 1 Minimum inhibitory concentration (MIC) breakpoints used for resistance classification of bacterial isolates (adapted from CLSI, 2018b)
2.9 | Data analysis

The questionnaire responses and antimicrobial susceptibility profiles of the bacterial isolates were coded and entered into SPSS statistics 25 (IBM) for descriptive analysis, i.e. frequencies and proportions were computed for all variables and presented as graphs and tables.

3 | RESULTS

3.1 | Farmer questionnaire survey

3.1.1 | Demographic profile of interviewed farmers

In total, 70 farmers were interviewed with the majority of them being men (80%, 56/70). More than half (59%, 37/63) of the farmers were over 45 years and majority of them (91%, 50/55) indicated that they had been rearing cattle for more than 5 years. Besides cattle, some of the farmers indicated rearing poultry (61%, 43/70), goats (24%, 17/70) and pigs (4%, 3/70), but none (0%, 0/70) reared sheep.

3.1.2 | Knowledge on antimicrobials and antimicrobial use practices of farmers

Only one farmer (1%) indicated being aware of what an antimicrobial is but could not give an example of one. Among the listed antimicrobials, the farmers indicated using Terramycin® (86%) and Hitet® (43%), both of which are oxytetracyclines. The farmers sourced medication mainly from the local animal clinic (60%) and an agricultural retailer (34%) in a neighbouring town (Table 2).

Twenty-nine per cent of the farmers kept treatment records for their cattle. Twenty-seven per cent of farmers indicated that they return expired drugs to place of purchase, 2% burn them, while the rest throw them away (Table 2).

Seventy-two per cent of the farmers indicated that they were aware of the importance of observing a withdrawal period after treating animals. Of these, 63% highlighted that it was important to do so because the drug will still be in the body of the animal and may affect them (Table 2).

Farmers were also asked if they had used drugs to treat for conditions such as diarrhoea, fever, coughing, mastitis, abscess or to prevent tick-borne diseases in the last year. Seven farmers (10%, 7/70) in total indicated having treated for one of the listed conditions. The farmers indicated treating for diarrhoea (6%, 4/70), fever (1%, 1/70), coughing (1%, 1/70) and for tick-borne disease prevention (1%). All seven farmers indicated that response to treatment was good. The farmers could not recall the drugs that they had used for treatment of the different ailments except for one farmer who indicated using Terramycin® (an oxytetracycline). In South Africa, Terramycin® is available as an over-the-counter medication without prescription.

| TABLE 2 Knowledge on antimicrobials and antimicrobial use practices of interviewed farmers |
|-----------------------------------------------|-----------------|-----------------|
| Do you know what an antimicrobial agent is   | Number of respondents | Percentage of respondents |
| No                                           | 69              | 99              |
| Yes                                          | 1               | 1               |
| Could give an example of an antimicrobial     | 0               | 0               |
| Antimicrobials farmers used in the last year |                 |                 |
| Terramycin®                                  | 60              | 86              |
| Hitet®                                       | 30              | 43              |
| Source of drugs                              |                 |                 |
| Corporative                                  | 23              | 34              |
| Local animal clinic                          | 40              | 60              |
| Veterinarian                                 | 6               | 9               |
| Animal health technicians                    | 0               | 0               |
| Villagers that sell drugs                    | 0               | 0               |
| Keep record of treatments given to their livestock |             |                 |
| Yes                                          | 19              | 29              |
| No                                           | 46              | 71              |
| How long do you use antibiotics for          |                 |                 |
| Until clinical signs stop                    | 3               | 5               |
| 3 days                                       | 16              | 24              |
| As indicated on the medicine use instructions| 1               | 2               |
| Until the drug is finished                   | 46              | 70              |
| Disposal of expired antimicrobials           |                 |                 |
| Throw in the bin or garbage pit             | 20              | 32              |
| Burn                                         | 1               | 2               |
| Throw in the toilet                         | 15              | 24              |
| Return to place of purchase                 | 17              | 27              |
| Throw away (not specified where)            | 9               | 14              |
| Do you know the importance of observing a withdrawal period |         |                 |
| Yes                                          | 48              | 72              |
| No                                           | 19              | 28              |
| Reasons given for observing a withdrawal period |                 |                 |
| Because the drug may affect humans          | 30              | 63              |
| To check if animal has fully recovered       | 15              | 31              |
| To prevent contracting the disease          | 1               | 2               |
| Simply because vets and AHTs instruct them to | 2               | 4               |
veterinary prescription. Therefore, efforts to identify if the farmers managed to buy prescription drugs without prescriptions as a follow-up question were thus futile. It is possible that the administered drugs the farmers failed to recall may have not been antimicrobials.

### 3.1.3 Knowledge on antimicrobial resistance (AMR)

Forty-two per cent of the farmers had heard about antimicrobial resistance with their main source being veterinarians and AHTs (82%). Of these, only one farmer (4%, 1/28) indicated that antimicrobial resistance involves microorganisms becoming resistant to treatment. However, the same farmer indicated that the body of the animal becomes resistant to treatment; hence, none of the farmers have a clear understanding of what antimicrobial resistance involves (Table 3). All the farmers indicated interest in learning more on prudent use of antimicrobials.

### 3.1.4 Animal health technicians and veterinarians’ interviews

Two AHTs were interviewed and both indicated that tetracyclines were the most commonly used antimicrobials in the community. Both rarely encountered cases of antimicrobial treatment failure but rated owner compliance with antimicrobial use instructions as poor. Both AHTs indicated underdosing by farmers as the main concern. One AHT also indicated the use of wrong routes of administration; inappropriate storage of drugs and use of expired drugs as areas of concern.

Three veterinarians (n = 3) contacted indicated that tetracyclines were commonly used while penicillins, sulphonamides and enrofloxacin were occasionally used.

### 3.2 Bacterial isolation and antimicrobial susceptibility

#### 3.2.1 Bacterial isolation

Seventy-nine per cent (79/100) of the cattle were culture positive for *E. coli* while 71% (71/100) were culture positive for *Enterococcus* species. One *Escherichia coli* and/or one *Enterococcus* were isolated from each culture-positive animal sample. A total of 150 bacterial isolates; 79 *Escherichia coli* and 71 enterococci were obtained. Forty-one per cent (29/71) of the enterococci isolates were not identified to species level. Of the speciated enterococci, *Enterococcus faecium* (79%; 33/42) was dominant followed by *E. faecalis* (10%; 3/42), *E. durans* (10%; 3/42) and *E. avium* (10%; 3/42).

![Figure 1: Antimicrobial susceptibility profile to five antimicrobials of *Enterococcus* isolates (n = 71) from healthy cattle](image)

#### 3.2.2 Antimicrobial susceptibility of *Enterococcus* species

Fifty-five per cent (39/71) of the *Enterococcus* isolates were resistant to at least one antimicrobial with enrofloxacin resistance (55%; 39/71) dominating followed by amoxycillin resistance (3%; 2/71). All (100%, 71/71) the enterococci isolates were susceptible to chlorotetacycline and vancomycin (Figure 1). Among the *Enterococcus* species, only *E. faecium* isolates (6%; 2/33) were resistant to amoxycillin while all species except for *E. faecalis* were resistant to enrofloxacin (Table 4). Among the antimicrobials tested against the enterococci isolates, vancomycin and chlorotetacycline had very narrow MIC distributions. Enrofloxacin had the widest MIC range which had a bimodal distribution with a cluster of susceptible/intermediate strains and a cluster of resistant strains (Figure 2). Two

| TABLE 3 Knowledge on antimicrobial resistance (AMR) of interviewed farmers |
|---------------------------------------------------------------|
| **Have you heard about AMR** *(n = 67)* | **Yes** | **No** |
| **Source of information on AMR** *(n = 28)* | **Health workers (veterinarians and AHTs)** | 23 | 82 |
| | **Television/radio** | 3 | 11 |
| | **Farmer’s day talk** | 2 | 7 |
| **Know any use practices that select for AMR development** *(n = 28)* | 0 | 0 |
| **With AMR, the body becomes resistant to treatment** *(n = 28)* | **Yes** | **No** |
| **With AMR, microorganisms become resistant to treatment** *(n = 28)* | **Yes** | **No** |
TABLE 4  Antimicrobial susceptibility profile of Enterococcus species (n = 71) from healthy cattle

| Species        | ∑n  | Amoxycillin n (%) | Enrofloxacin n (%) | Chlortetracycline n (%) | Vancomycin n (%) | Erythromycin n (%) |
|----------------|-----|-------------------|--------------------|------------------------|------------------|-------------------|
|                |     | S     I      R    | S   I      R     | S       I      R      | S     I      R    | S     I      R     |
| E. faecium     | 33  | 31 (94) 0    2 (6) | 2 (6) 1 (3) 30 (91) | 33 (100) 0 0   | 33 (100) 0 0 | 12 (36) 21 (64) 0 |
| E. faecalis    | 3   | 3 (100) 0    0 0 | 2 (67) 1 (33) 0 0 | 3 (100) 0 0    | 3 (100) 0 0 | 27 (93) 2 (7) 0  |
| Enterococcus  | 29  | 29 (100) 0   0 0 | 18 (62) 8 (28) 3 (10) | 29 (100) 0 0  | 29 (100) 0 0 | 1 (33) 2 (67) 0  |
| E. avium      | 3   | 3 (100) 0    0 0 | 1 (33) 0 2 (67) | 3 (100) 0 0   | 3 (100) 0 0 | 2 (67) 1 (33) 0  |
| E. durans     | 3   | 3 (100) 0    0 0 | 0 0 3 (100)       | 3 (100) 0 0   | 3 (100) 0 0 | 2 (67) 1 (33) 0  |

Note: ∑n = Total number of isolates tested.

n = Number of isolates tested.

(%) = Percentage of isolates.

S, susceptible; I, intermediate; R, resistant, n, number of isolates.

FIGURE 2  Minimum inhibitory concentrations of five antimicrobials for Enterococcus isolates (n = 71) from healthy cattle

FIGURE 3  Antimicrobial susceptibility to five antimicrobials of E. coli isolates from healthy cattle

3.2.3  Antimicrobial susceptibility of E. coli isolates

Twenty-seven per cent (21/79) of the E. coli isolates were resistant to at least one antimicrobial. The highest level of resistance detected was against colistin (16%, 12/79). All (100%, 79/79) of the isolates were susceptible to gentamicin (Figure 3). All of the antimicrobials tested except for enrofloxacin (Figure 4) Seven phenotypic resistance patterns were detected in the E. coli isolates (Table 5). Amoxycillin-chlortetracycline resistance was the dominant co-resistance phenotype. No multidrug resistant strains were detected.

The minimum inhibitory concentrations of the antimicrobials for the ATCC control strains fell within the CLSI range.

Note: ∑n = Total number of isolates tested.

n = Number of isolates tested.

(%) = Percentage of isolates.

S, susceptible; I, intermediate; R, resistant, n, number of isolates.
4 | DISCUSSION

4.1 | Questionnaire surveys

Containing antimicrobial resistance requires the input of everyone in society (World Health Organisation, 2015b) with farmers being among the individuals with a significant role to play. In this study, only one farmer (1%) knew what an antimicrobial is, despite many of them using antimicrobials. This ignorance is concerning as farmers might not take the necessary precautions when using antimicrobials or may use them injudiciously. A clear understanding of how antimicrobials function and when their use is indicated is a prerequisite for judicious use behaviour (Ancillotti et al., 2018). Failure of the farmers in this study to describe when their use is indicated is a prerequisite for judicious use behaviour. Farmers must be encouraged to keep good records as they are an important means of monitoring both disease burden and antimicrobial use on the farm (Speksnijder, Mevius, Bruschke, & Wagenaar, 2015). Safe disposal of antimicrobials is also imperative to avoid environmental contamination and increased antimicrobial resistance selection pressure (Bound & Voulvoulis, 2005).

Despite tetracyclines being the popular antimicrobial used by the farmers, we observed low levels of resistance to tetracyclines. Other investigators have made similar findings among small-scale farmers (Mainda et al., 2015; Mubita et al., 2008). One of the limitations of this study was that we did not investigate the volumes and frequency of antimicrobial use. Perhaps the use of the tetracyclines by the farmers in our study was not frequent enough to exert adequate selective pressure for resistance development.

All the farmers in this study sourced their veterinary drugs from legally established distributors. This is a positive finding since antimicrobials sold by legal distributors in South Africa are expected to be registered with the relevant authorities and are therefore quality assured (Eagar et al., 2012). This significantly reduces the risk of resistance development due to use of poor-quality agents. Small-scale dairy farmers in Zambia (91%) and rural Peru (87.8%) sourced their drugs from veterinarians and veterinary drug stores (Mainda et al., 2015; Redding et al., 2014). Adequate training of veterinary drug salesmen on animal health and disease management, and prudent drug use is thus critical since these people may at times be the only animal health informants at the farmers’ disposal.

A clear understanding of antimicrobial resistance and its consequences in animals and humans is a significant motivator for prudent use of antimicrobials (Eltayb, Barakat, Marrone, Shaddad, & Stålsby Lundborg, 2012; Sirdar, Picard, Bisschop, & Gummow, 2012). Therefore, it is worrying that half of the farmers were not aware of the public health importance of observing withdrawal periods. In addition, some of the withdrawal periods observed by the farmers fell short of the recommended periods for the tetracyclines they indicated using.

Among the farmers (70%) who indicated using antimicrobials until the bottle is empty, it is possible that some of them were referring to use of an antimicrobial or even other veterinary agents in general as opposed to duration of antimicrobial use when treating a sick animal. Nonetheless, poor compliance with antimicrobial use instructions, poor record keeping of treated animals and improper disposal of expired antimicrobial drugs by farmers in this study are likely to increase the development of antimicrobial resistance (Bound & Voulvoulis, 2005). The use of incorrect doses and routes of administration is also likely to increase the risk of the development of resistance and exposure of the public to antimicrobial residues (Khatun et al., 2018). Farmers must be encouraged to keep good records as they are an important means of monitoring both disease burden and antimicrobial use on the farm (Speksnijder, Mevius, Bruschke, & Wagenaar, 2015).
Although some farmers indicated hearing about antimicrobial resistance, they may have been referring to drug resistance in general given that the farmers indicated not being aware what an antimicrobial is. Antimicrobial resistance awareness campaigns should be developed with the help of veterinarians and AHTs working in the area as farmers recognise them as their main source of information on antimicrobial use.

4.2 | Antimicrobial susceptibility of bacterial isolates

4.2.1 | *Enterococcus* species

*Enterococcus faecium* was the most common *Enterococcus* species isolated in this study. Similar finding has been reported in cattle in Nigeria (Ngbede, Raji, Kwanashie, Kwada, & Kwaga, 2017). In contrast, *E. hirae* was the most common species in dairy cattle in a previous study in South Africa (Tanih, 2016). The differences between studies may be related to diet and variation in the environmental microbiomes to which the different cattle populations are exposed (Anderson, Parrish, Akhtar, Zurek, & Hirt, 2008) as well as use of different culture media (Jackson, Fedorka-Cray, Jackson-Hall, & Hiott, 2005). Polymerase chain reaction would have been helpful in identifying the non-specified enterococci to species level.

Low levels of resistance to the tested antimicrobials were detected among the enterococci with the exception of enrofloxacin. Similarly, high resistance to enrofloxacin (90%) was observed in cattle in the South African National Veterinary Surveillance and Monitoring Program (SANVAD) (van Vuuren et al., 2007). In contrast, higher levels of resistance to tetracycline (26.7%–100%), erythromycin (20%–88.9%) and vancomycin (20%–33%) were detected in South Africa at selected commercial dairy farms (Tanih, 2016) and in the SANVAD. In addition, resistance to ampicillin (40% for *E. faecium*) was high in the SANVAD (van Vuuren et al., 2007).

4.2.2 | *Escherichia coli*

The proportion of faecal samples from which *E. coli* was isolated in this study was lower than expected considering that it is one of the dominant commensal flora of the gastrointestinal tract of cattle. This could be due to the drying of swabs due to lack of transport medium (Centre for Disease Control & Prevention, 1994).

The level of resistance to tetracycline, amoxycillin, enrofloxacin and gentamicin in *E. coli* isolates in this study was generally low. Similarly, low levels of resistance (0.8%–4.2%) to these antimicrobials were detected in the SANVAD with the exception of tetracycline resistance which was higher (33.6%) (van Vuuren et al., 2007). Our results were also similar to the low levels of resistance reported in cattle in Zambia (Mainda et al., 2015; Mubita et al., 2008).

The level of colistin resistance detected in the *E. coli* isolates was unexpected considering that the study focused on a rural communal farming area. However, colistin resistance has previously been detected in *E. coli* isolates from hosts not previously exposed to colistin or any antimicrobial treatment (Bachiri et al., 2018; Liakopoulos, Mevius, Olsen, & Bonnedahl, 2016; Ruzauskas & Vaskeviciute, 2016).

Colistin has a tendency to adsorb to laboratory plastic ware resulting in reduced availability of the antimicrobial in assays (Humphries, 2015; Karvanen, Malmberg, Lagerbäck, Friberg, & Cars, 2017). This might have falsely elevated minimum inhibitory concentrations (MICs) resulting in overestimation of resistance levels in this study. Furthermore, lack of an intermediate susceptibility category for colistin might have also led to false resistant categorisation of isolates with minimum inhibitory concentrations close to the resistance breakpoint (Matuschek, Åhman, Webster, & Kahlmeter, 2018). Nonetheless, the high level of colistin resistance observed in this study warrants further molecular investigation.

The most common co-resistance phenotype in the *E. coli* isolates in this study was amoxycillin–chlorotetracycline resistance as was the case in the study in dairy cattle in Zambia (Mainda et al., 2015). This co-resistance may be due to resistance genes being borne on the same mobile genetic elements and these can disseminate rapidly resulting in increased resistance levels.

4.3 | Study limitations

Despite the important findings from the questionnaire survey, it has some shortcomings. The responses in this questionnaire survey were self-reported and thus may be subject to recall bias (Caudell et al., 2017). Ignorance of what antimicrobials are potentially biased some of the responses in that perhaps the farmers were in some instances making reference to drugs in general as opposed to antimicrobials specifically. The small cattle sample size may have influenced the resistance levels detected in this study.

5 | CONCLUSION

The level of antimicrobial resistance in this study was generally low. Of concern is the level of colistin-resistant *E. coli* isolates detected. Further molecular investigation is warranted to check if the detected colistin resistance is plasmid mediated. Over-the-counter availability of antimicrobials must be accompanied by tailor-made farmer education programmes that raise awareness on prudent antimicrobial use and antimicrobial resistance to help promote responsible antimicrobial use among farmers. However, it is important to note that this awareness alone does not guarantee behavioural change. These awareness campaigns should be supported by other interventions including primary animal health training programmes for farmers and policy changes.
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CONFLICT OF INTEREST
The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTION
Charlotte Ropafadzo Mupfunya: Investigation; Methodology; Writing-original draft; Writing-review & editing. Daniel Neneke Qekwana: Methodology; Supervision; Writing-review & editing. Vinny Naidoo: Conceptualization; Funding acquisition; Methodology; Supervision; Writing-review & editing.

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ORCID
Charlotte Ropafadzo Mupfunya https://orcid.org/0000-0002-7160-0914
Daniel Neneke Qekwana https://orcid.org/0000-0002-4609-5151
Vinny Naidoo https://orcid.org/0000-0003-2740-5983

REFERENCES
Aarestrup, F. M. (2015). The livestock reservoir for antimicrobial resistance: A personal view on changing patterns of risks, effects of interventions and the way forward. Philosophical Transactions of the Royal Society B Biological Sciences, 370(1670), 20140085. https://doi.org/10.1098/rstb.2014.0085
Ancillotti, M., Eriksson, S., Veldwijk, J., Fahlquist, J. N., Andersson, D. I., & Godskesen, T. (2018). Public awareness and individual responsibility needed for judicious use of antibiotics: A qualitative study of public beliefs and perceptions. BMC Public Health, 18(1), 1153. https://doi.org/10.1186/s12889-018-6047-8
Anderson, J. F., Parrish, T. D., Akhtar, M., Zurek, L., & Hirt, H. (2008). Antibiotic resistance of enterococci in American Bison (Bison bison) from a nature preserve compared to that of enterococci in pastured cattle. Applied and Environmental Microbiology, 74(6), 1726-1730. https://doi.org/10.1128/AEM.02164-07
Aubry, P., & Geale, D. W. (2011). A review of Bovine anaplasmosis. Transboundary and Emerging Diseases, https://doi.org/10.1111/j.1865-1682.2010.01173.x
Bachiiri, T., Lalaoui, R., Bakour, S., Allouache, M., Belkebla, N., Rolain, J. M., & Touati, A. (2018). First report of the plasmid-mediated colistin resistance gene mcr-1 in Escherichia coli ST405 isolated from wildlife in Bejaia, Algeria. Microbial Drug Resistance. 24(7):890–895. https://doi.org/10.1089/mdr.2017.0026
Berrian, A. M., Van Rooyen, J., Martinez-Lopez, B., Knobel, D., Simpson, G. J., Wilkes, M. S., & Conrad, P. A. (2016). One Health profile of a community at the wildlife-domestic animal interface, Mpumalanga, South Africa. Preventive Veterinary Medicine, 130, 119-128. https://doi.org/10.1016/j.prevetmed.2016.06.007
Bound, J. P., & Voulovouinis, N. (2005). Household disposal of pharmaceuticals as a pathway for aquatic contamination in the United Kingdom. Environmental Health Perspectives, 113(12), 1705-1711. https://doi.org/10.1289/ehp.8315
Caprioli, A., Busani, L., Martel, J. L., & Helmuth, R. (2000). Monitoring of antibiotic resistance in bacteria of animal origin: Epidemiological and microbiological methodologies. International Journal of Antimicrobial Agents, 14(4), 295–301. https://doi.org/10.1016/s0924-8579(00)00140-0
Caudell, M. A., Quinlan, M. B., Subbiah, M., Call, D. R., Roulette, C. J., Roulette, J. W., ... Quinlan, R. J. (2017). Antimicrobial use and veterinary care among agro-pastoralists in Northern Tanzania. PLoS One, 12(1), e0170328. https://doi.org/10.1371/journal.pone.0170328
Centre for Disease Control and Prevention. (1994). E. coli O157:H7: Procedure for Isolation and Identification from Stool Specimens. Retrieved from https://wonder.cdc.gov/wonder/preguid/p0000445/p0000445.asp
Chauhan, A. S., George, M. S., Chatterjee, P., Lindahl, J., Grace, D., & Kakkar, M. (2018). The social biography of antibiotic use in smallholder dairy farms in India. Antimicrobial Resistance and Infection Control, 7(1), 1-13. https://doi.org/10.1186/s13756-018-0354-9
Choopa, C. N. (2016). Diagnosis of tick-borne diseases in cattle in Bushbuckridge Mpumalanga South Africa and identification of Theileria parva carriers (Doctoral dissertation, University of Pretoria).
Clinical and Laboratory Standards Institute (CLSI). (2018b). Performance standards for antimicrobial susceptibility testing (28th ed.). CLSI Supplement M100. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute.
Clinical and Laboratory Standards Institute (CLSI). (2018a). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals (4th ed.). CLSI VET08. Wayne, Pennsylvania. Retrieved from http://vet01s.edapvedocs.info
Eagar, H., Swan, G., & Van Vuuren, M. (2012). A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. Journal of the South African Veterinary Association, 83(1), 15–23. https://doi.org/10.4102/jsava.v83i1.16
Eltyab, A., Barakat, S., Marrone, G., Shaddad, S., & Stalsby Lundborg, C. (2012). Antibiotic use and resistance in animal farming: a quantitative and qualitative study on knowledge and practices among farmers in Khartoum, Sudan. Zoonoses and Public Health, 59(5), 330–338. https://doi.org/10.1111/j.1863-2378.2012.01458.x
Franklin, A., Acar, J., Anthony, F., Gupta, R., Nicholls, T.J., Tamura, Y., ... Costarica, M.L. (2001). Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. Revue Scientifique et Technique de l’OIE, 20, (3), 859–870. http://dx.doi.org/10.1289/ehp.8315
Henton, M. M., Eagar, H. A., Swan, G. E., & van Vuuren, M. (2011). Part VI. Antibiotic management and resistance in livestock production. South African Medical Journal, 101(8), 583–586.
Humphries, R. M. (2015). Susceptibility testing of the polymyxins: Where are we now? Pharmacotherapy, 35(1), 22–27. https://doi.org/10.1002/phar.1505
Jackson, C. R., Fedorka-Cray, P. J., Jackson-Hall, M. C., & Hiott, L. M. (2005). Effect of media, temperature and culture conditions on the species population and antibiotic resistance of enterococci from broiler chickens. Letters in Applied Microbiology, 41(3), 262–268. https://doi.org/10.1111/j.1477-755X.2005.01749.x
Karvanen, M., Malmberg, C., Lagerbäck, F., Friberg, L. E., & Cars, O. (2017). Colistin is extensively lost during standard in vitro experimental conditions. Antimicrobial Agents and Chemotherapy, 61(11), 1–9. https://doi.org/10.1128/AAC.00857-17
Katakweba, A. A. S., Mtambo, M. M. A., Olsen, J. E., & Muhairwa, A. P. (2012). Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. Livestock Research for Rural Development, 24(10), 170.

Khatun, R., Jabbar Howlader, A., Ahmed, S., Islam, N., Alam, K., Haider, S., ... Hasan, M. A. (2018). Validation of the declared withdrawal periods of antibiotics. Universal Journal of Public Health, 6(1), 14–22. https://doi.org/10.13189/ujph.2018.060103

Liakopoulos, A., Mevius, D. J., Olsen, B., & Bonnedahl, J. (2016). The colistin resistance mcr-1 gene is going wild. Antimicrobial Chemotherapy, 71(8), 2335–2336. https://doi.org/10.1093/jac/dkw262

Midorikas, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection, 18(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Mainda, G., Bessell, P. R., Muma, J. B., McAteer, S. P., Chase-Topping, M., Meissner, H. H., Scholtz, M. M., & Palmer, A. R. (2013). Sustainability of livestock for rural development. 24(10), 170.

Monea, T., Maburutse, B. E., Mbiriri, D. T., & Kashangura, M. T. (2012). Veterinary pharmacovigilance: A South African perspective. In S. C. M. (Ed.), Veterinary pharmacovigilance: Supporting Information section. Additional supporting information may be found online in the Supporting Information section.

Naidoo, V. Antimicrobial use practices and resistance in indicator bacteria in communal cattle in the Mnisi community, Mpumalanga, South Africa. Vet Med Sci. 2021;7:112–121. https://doi.org/10.1002/vms3.334

Redding, L. E., Cubas-Delgado, F., Sammel, M. D., Smith, G., Galligan, D. T., Levy, M. Z., & Hennessy, S. (2014). Understanding the use of antibiotics on small dairy farms in rural Peru. Preventive Veterinary Medicine, 113(1), 88–95. https://doi.org/10.1016/j.prevetmed.2013.10.012

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Vila, J. (2015). The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections, 6, 22–29. https://doi.org/10.1016/j.nmni.2015.02.007

Ruzauskas, M., & Vaskeviuciute, L. (2016). Detection of the mcr-1 gene in Escherichia coli prevalent in the migratory bird species Larus argentatus. Journal of Antimicrobial Chemotherapy, 71(8), 2333–2334. https://doi.org/10.1093/jac/dkw245

Sirdar, M. M., Picard, J., Bisschop, S., & Gumbow, B. (2012). A questionnaire survey of poultry layer farmers in Khartoum State, Sudan, to study their antimicrobial awareness and usage patterns. Ondersteypoort Journal of Veterinary Research, 79(1), 1–8. https://doi.org/10.4102/ojvr.v79i1.361

Speksnijder, D. C., Mevius, D. J., Bruschke, C. J. M., & Wagenaar, J. A. (2015). Reduction of veterinary antimicrobial use in the Netherlands. The Dutch success model. Zoonoses and Public Health, 62(s1), 79–87. https://doi.org/10.1111/zph.12167

Tanih, G. N. (2016). Genotypic and phenotypic characterization of enterococci from cow dung and environmental water sources in three selected dairy farms in Amathole District (Doctoral dissertation, University of Fort Hare)

van Vuuren, M., Picard, J., & Greyling, J. (2007). SANVAD 2007: South African national veterinary surveillance and monitoring programme for resistance to antimicrobials drugs, University of Pretoria, Faculty of Veterinary Science, Department of Veterinary Tropical Diseases.

Varga, C., Rajič, A., McFall, M. E., Reid-Smith, R. J., Deckert, A. E., Pearl, D. L., ... McEwen, S. A. (2008). Comparison of antimicrobial resistance in generic Escherichia coli and Salmonella spp. cultured from identical fecal samples in finishing swine. Canadian Journal of Veterinary Research, 72(2), 181–187.

World Health Organisation (WHO) (2015a). Global action plan on antimicrobial resistance. Retrieved from http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf

World Health Organisation (WHO). (2015b). Antibiotic resistance: Multi-country public awareness survey. Retrieved from http://www.who.int/drugresistance/documents/baselinesurvey2015/en/

Yunker, C. E. (1996). Heartwater in sheep and goats: A review. Veterinary Medicine (Copenhagen, Denmark), 91(2), 146–151.