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DOI: 10.1002/vms3.1315

ORIGINAL ARTICLE

WILEY

Prevalence of colistin resistance and antibacterial resistance in commensal *Escherichia coli* from chickens: An assessment of the impact of regulatory intervention in South Africa

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Abstract

Background: Antimicrobial resistance (AMR) is a global health problem largely due to the overuse of antimicrobials. In recognition of this, the World Health Assembly in 2015 agreed on a global action plan to tackle AMR. Following the global emergence of the *mcr-1*-associated colistin resistance gene in the livestock industry in 2016, several countries including South Africa restricted the veterinary use of colistin as the gene threatens the clinical utility of the drug. This study is a follow-up to the restriction in place in order to evaluate the impact of such policy adoption.

Objective: To assess the prevalence of antibacterial resistance (ABR), and the *mcr-1* colistin resistance gene in broiler chicken over a 2-year period, as a follow-up to the veterinary ban on colistin use in South Africa.

Methods: A total of 520 swab samples were obtained during 2019 (March–April) and 2020 (February–March), from healthy broiler chicken carcasses ($n = 20$) and chicken droppings in transport crates ($n = 20$) at various poultry abattoirs ($N = 7$) in the Gauteng province of South Africa. *Escherichia coli* organisms were isolated and subjected to a panel of 24 antibacterials using the MicroScan machine. Screening for *mcr-1* colistin resistance gene was undertaken using PCR.

Result: Four hundred and thirty-eight (438) *E. coli* strains were recovered and none demonstrated phenotypic resistance towards colistin, amikacin, carbapenems, tigecycline and piperacillin/tazobactam. The *mcr-1* gene was not detected in any of the isolates tested. Resistances to the aminoglycosides (0%–9.8%) and fluoroquinolones (0%–18.9%) were generally low. Resistances to ampicillin (32%–39.3%) and trimethoprim/sulphamethoxazole (30.6%–3.6%) were fairly high. A significant ($p < 0.05$) increase in cephalosporins and cephamycin resistance was noted in the year 2020 (February–March) when compared with the year 2019 (March–April).

Conclusion: The absence of *mcr*-1 gene and colistin resistance suggests that mitigation strategies adopted were effective and clearly demonstrated the significance of regulatory interventions in reducing resistance to critical drugs. Despite the drawback in regulatory framework such as free farmers access to antimicrobials OTC and a dual registration system in place, there is a general decline in the prevalence of ABR when

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the present data are compared with the last national veterinary surveillance on AMR (SANVAD 2007). To further drive resistance down, mitigation strategies should focus on strengthening regulatory framework, the withdrawal of OTC dispensing of antimicrobials, capping volumes of antimicrobials, banning growth promoters and investing on routine surveillance/monitoring of AMR and antimicrobial consumption.

KEYWORDS
antimicrobial resistance, colistin, *E. coli*, *mcr-1*, South Africa

1 | INTRODUCTION

The global burden of antimicrobial resistance (AMR) is well documented, with the livestock industry's use of antimicrobials considered a major contributor (Office International des Epizooties [OIE], 2015; Van Boeckel et al., 2015, 2019). Veterinary consumption of antimicrobials in South Africa represents two thirds of the total volume of antimicrobials used to manage infections (National Department of Health [NDoH], 2022). This is similar to the global livestock industry which accounts for about two thirds of the overall global antimicrobial consumption (SANDH, 2018; Van Boeckel et al., 2019). More importantly, recent projections demonstrate that this high volume is expected to have risen by 67% in the year 2030 together with an alarming rise in South Africa estimated at 99% (Van Boeckel et al., 2015). In light of this, emphasis is now geared towards reducing local and global consumption of antimicrobials.

In the World Health Assembly of 2015, the World Health Organization (WHO) highlighted several key areas where effort should be directed for any meaningful impact such as; strengthening knowledge and evidence base via surveillance and research, raising public awareness on AMR, reducing the need for antimicrobial use (AMU) by adequate preventive measures, and optimizing AMU among others (Mendelson & Matsoso, 2015; WHO, 2015). In conjunction with the WHO's recommendation for AMR awareness initiatives, several countries including the USA, Sweden, Japan, the Netherlands, Denmark and Columbia have established national limits on total volumes of veterinary antimicrobials consumed (Bandyopadhyay & Samanta, 2020). As a result, the aforementioned countries' use of antimicrobials for livestock growth promotion has been effectively phased out.

In South Africa, the significance of routine surveillance and monitoring of AMR and antimicrobial consumption in guiding veterinary antimicrobial choices and checkmating the spread of AMR has long been recognized (Nel et al., 2004). This recognition led to the first government-sponsored expert group meeting held at Durban in 2003, where an earlier proposal to establish a standardized surveillance and monitoring programme in South Africa was re-emphasized (Van Vuuren et al., 2007). Subsequently, in 2005/2006, the only national veterinary AMR surveillance conducted so far in South Africa was completed (Van Vuuren et al., 2007). Similarly, in 2012, an attempt was made to collate data on volumes of veterinary antimicrobials consumed in South Africa (Eagar et al., 2012). More recently, the NDoH published the total volumes of veterinary antimicrobials consumed in South Africa for the years 2014, 2015 and 2021 (NDoH, 2022; SANDH, 2018). The documents were, however, lacking a comprehensive veterinary AMR surveillance data precluding any definite conclusion on the local impact of AMU. Nonetheless, there was a definite commitment with policy makers charting out a national multidisciplinary strategic framework to combat AMR (NDOH, DAFF, 2018). Parts of its objectives among others include surveillance, strengthening regulations and enforcement mechanism so as to ensure appropriate veterinary use of antimicrobials (NDOH, DAFF, 2018).

In line with these government objectives, the present study was designed to measure the prevalence of antibacterial resistance (ABR) in broiler chicken farms in the Gauteng province of South Africa, by monitoring resistance present in organisms cultured from chickens presenting at the abattoir for slaughter over a 2-year period. Domestic chickens were selected as the target species, as they are the cheapest source of meat, which as a result is the most consumed animal protein in South Africa (Davids & Meyer, 2017). More importantly, it is critical to acknowledge the volume of antimicrobials used in the chicken for consumption industry (Andrew Selaledi et al., 2020; Henton et al., 2011). In this regard, the foodborne transmission of AMR is of specific concern, and sampling of carcasses at the abattoir would provide valuable information on 'slaughter hygiene and level of contamination and cross contamination of meat (OIE, 2015)'. Similarly, faecal samples collected at the abattoir would demonstrate the prevalence of resistant *Escherichia coli* entering the environment from slaughtered animals (OIE, 2015).

More specifically, the study looked at the prevalence of the *mcr-1* colistin resistance gene in the said samples collected. This gene emerged in South African livestock industry in 2016 threatening the clinical utility of colistin, thus necessitating a restriction on veterinary colistin use (Hassan et al., 2021; Perreten et al., 2016; SAVC, 2016). This is a follow-up to the restriction in place so as to evaluate the impact/justification of such policy adoption.

2 | MATERIALS AND METHODS

2.1 | Ethics approval

Approval for this study was obtained from the research committee of the Faculty of Veterinary Science, University of Pretoria

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(V098-17); and the South African Department of Agriculture, Forestry and Fisheries (DAFF) (12/11/1/1/9).

2.2 | Study population and sampling

Random swab samples were collected aseptically from dressed chicken carcasses ($n = 20$) and transport crates ($n = 20$) per poultry abattoir ($N = 7$) within Gauteng province of South Africa. Sampling was undertaken in the months of March and April 2019 and repeated in February and March 2020. Abattoirs sampled were not randomly selected as permission to access facility needed to be granted. An overall total of 520 samples were collected as repeat sampling in 2020 was short of one abattoir due to COVID-related regulations. The sample size utilized was extrapolated based on an expected low (i.e. 10%) prevalence of ABR, around a confidence level of 95% with a 5% precision (OIE, 2015). Additionally, 20 faecal swab samples were collected from 20 broiler pens that housed antibiotic-free birds in a controlled environment to serve as control. Samples were conveyed in transport media (Amies) on ice to prevent the desiccation of organism.

The sampled province covers an approximate land mass area of 18,176 km^2 producing about 10% of total broiler produced (i.e. 1.7 million tons) in the country (DAFF, 2020). The coordinates of sample sites were measured using the mobile free My GPS Coordinates, version 2.14, and were plotted on map using ArcGIS Pro software version 2.9 (Esri Inc.) (Figure 1).

2.3 | Isolation

All samples were processed within 5 h of collection. Briefly, samples were plated unto MacConkey agar and incubated aerobically for 24 h at 37°C. Subsequently, resulting single pinkish colonies were picked and grown on Eosin Methylene Blue agar for 24 h at 37°C. Isolates with a greenish metallic sheen appearance were presumptively identified as *E. coli* and stored at −20°C.

2.4 | Isolate characterization and minimum inhibitory concentration (MIC) measurement

The isolates identification and antibacterial susceptibility testing were undertaken using an automated system, the WalkAway 40 *plus* MicroScan machine (Beckman Coulter Inc.) following manufacturer's instructions. Fresh overnight grown cultures on 5% sheep blood agar were analysed using the prompt method of inoculation. The minimum inhibitory concentration (MIC) for each strain was evaluated for amikacin, ampicillin, aztreonam, cefotaxime, cefoxitin, ceftazidime, cefuroxime, ciprofloxacin, colistin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, nalidixic acid, nitrofurantoin, norfloxacin, piperacillin/tazobactam, tigecycline, trimethoprim/sulphamethoxazole, tobramycin, cefepime, ampicillin/sulbactam and amoxicillin/clavulanate antibacterials. The MICs for each individual drug compound required to kill 90% of the bacterial strains (MIC_{90}) were compared with their respective breakpoint (BP). Isolates with ≥3 twofold decrease in MICs to ceftazidime or cefotaxime in the presence of a fixed concentration of clavulanic acid (4 μg/mL) vs. its MIC when tested alone is classified as ESBL producer (NCCLS, 2003; Stürenburg et al., 2004). A *mcr-1*-positive colistin resistant clinical *E. coli* strain recovered from human UTI (kindly donated by Prof Marleen Kock) and *E. coli* ATCC 25922 were included for quality control. The epidemiological cut-off values provided by The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) guidelines were used for interpretation (CLSI, 2018; EUCAST, 2019). This became necessary because panels used for analysis were not adapted for EUCAST interpretation with certain drugs.

2.5 | Screening for the presence of mcr-1 gene

Direct colony PCR was carried out on 100 randomly selected *E. coli* strains each from the 2019 to 2020 collections, respectively. This became necessary as the *mcr-1* gene has often been shown to exist unexpressed (Fernandes et al., 2016; Hassan et al., 2021; Lentz et al., 2016; Terveer et al., 2017). For each year of collection, 50 strains were selected per source, that is chicken carcasses and faeces. The online research randomizer (version 4.0) computer software was utilized for the selection as all strains were serially numbered. The programme generated random numbers identifying strains to be included. Colonies served as DNA template in the reaction mixtures, with 12.5 μL of DreamTaq Green 2× Master Mix (Thermo Fisher Scientific), 6.5 μL nuclease-free water and 0.5 μL of each primer solution (F: 5′CGGTCAGTCCGTTTGTTC3′ and R: 5′CTTGGTCGGTCTGTAGGG3′) specific for the *mcr-1* gene. The thermo-cycling condition was maintained at 94°C 15 min + 25 × (94°C 30 s + 58°C 90 s + 72°C 60 s) + 72°C 10 min, using a MiniAmp Plus Thermal Cycler (Thermo Fisher Scientific). Resulting amplicons were subjected to agarose gel electrophoresis using 1.5% gel in 1× TBE with ethidium bromide. The gels ran for 90 min at 90 V before visualizing the bands (Cavaco et al., 2016). The PCR product expected for the *mcr-1* gene was expected to have 309 base pairs.

2.6 | Statistical analysis

Descriptive analysis of categorical variables and their 95% confidence interval were computed using SPSS version 23 (IBM SPSS Statistics for Windows, version 23.0). Proportions of ABR were compared between years and sources of samples using chi-square with the level of significance set at $\alpha < 0.05$. Fisher's exact test and Yate's continuity correction were used in cases of small expected cell sizes (McDonald, 2009).

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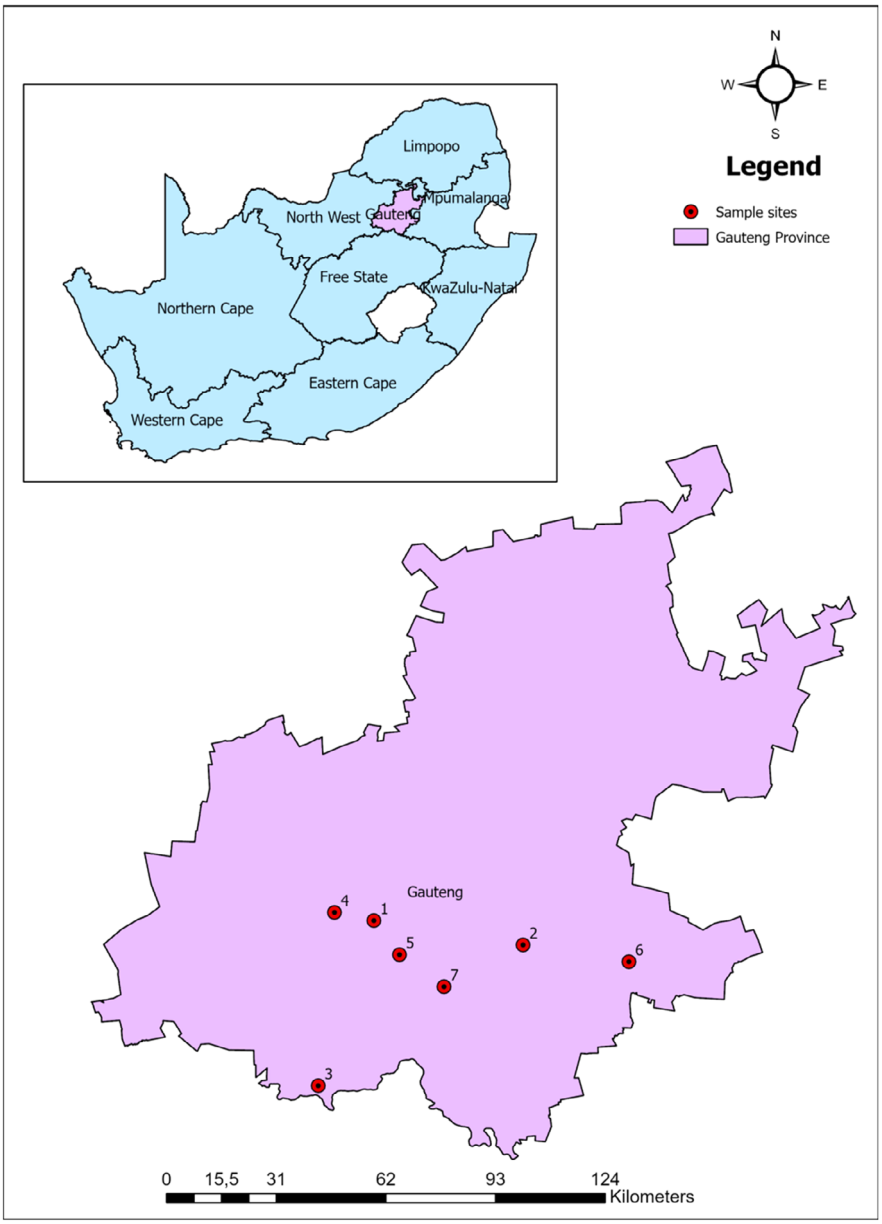

FIGURE 1 A map illustration showing sites of sampling in Gauteng province of South Africa.

3 | RESULTS

3.1 | Recovered strains

Of the 520 swab samples collected, 438 (84.2%) *E. coli* strains were recovered for subsequent antibiogram analysis (Table 1).

3.2 | Antibacterial susceptibility testing

3.2.1 | Colistin susceptibility testing and mcr-1 gene evaluation

Following colistin evaluation, no strain was phenotypically resistant to the said drug (Table 2). Of the 200 strains screened, none carried the *mcr-1* gene.

3.2.2 | General antibiogram testing

Following antibiogram analysis, the proportions of resistance to ampicillin (32%–39.3%) and trimethoprim/sulphamethoxazole (30.6%–3.6%) were quite high irrespective of sample source or year when compared to the other antibacterials tested. This latter observation was common irrespective of source and/or year of sampling. No resistance was detected towards amikacin, imipenem, ertapenem, meropenem, tigecycline and piperacillin/tazobactam for all strains tested. Resistances to the cephalosporins and cephamycin were generally low during 2019 but increased significantly ($p < 0.05$) in the subsequent year. This is also true for amoxicillin/clavulanate. Extended spectrum β-lactamase production was noted in 9.8% ($n = 43$) of the strains. Of all the quinolones tested, the proportions of resistance to nalidixic acid (47.5%–21.4%) appear to be fairly high, and the others (i.e. ciprofloxacin, levofloxacin and norfloxacin) demonstrated low level

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TABLE 1 Distribution of *Escherichia coli* isolates per sources of samples.

	Environment (% (*n*/*N*))		Carcasses (% (*n*/*N*))		Total
Site	2019	2020	2019	2020	% (*n*/*N*)
1	15.2 (17/112)	–	12.3 (15/122)	–	13.7 (32/234)
2	13.4 (15/112)	20.4 (20/98)	15.6 (19/122)	17 (18/106)	16.4 (72/438)
3	16.1 (18/112)	18.4 (18/98)	13.1 (16/122)	18.9 (20/106)	16.4 (72/438)
4	11.6 (13/112)	18.4 (18/98)	16.4 (20/122)	18.9 (20/106)	16.2 (71/438)
5	15.2 (17/112)	9.2 (9/98)	14.8 (18/122	16.0 (17/106)	13.9 (61/438)
6	17 (19/112)	18.4 (18/98)	14.8 (18/122)	14.2 (15/106)	16.0 (70/438)
7	11.6 (13/112)	15.3 (15/98)	13.1 (16/122)	15.1 (16/106)	13.7 (60/438)

of resistance (18.9%–2%). The proportions of resistance on the aminoglycosides (i.e. gentamicin and tobramycin) were comparatively low as demonstrated in Table 2. For all antibacterials, no significant differences ($p > 0.05$) were noted when data between the two different sources were compared by year (Table 2).

The MICs for each individual drug compound required to kill 90% of the bacterial strains (MIC_{90}) were compared with their respective BP (Table 3). For ampicillin, the MIC_{90} were all above the BP (Table 3). The MIC_{90} for the cephalosporins and cephamycin were below their BPs in 2019; however, in the subsequent year, the MIC_{90}s were above their BP with exception for cefoxitin where the MIC_{90} rose to the BP value. The MIC_{90} for nalidixic acid, ciprofloxacin and norfloxacin were largely above their BPs. For most other remaining drug compounds tested, the MIC_{90}s remained below their BP during the years of sampling.

Looking at the MIC frequency distribution table (Table 3), our data showed a clear distinction between bacterial sub-populations based on strains response to antibacterial drugs tested. Notwithstanding, most strains were regarded as wild type as they demonstrated MICs below the epidemiological BPs. In this regard, the minimum drug concentrations required to kill the strains in question were within normal for the species wild population. The results did, however, indicate trends for resistance development, with drug compounds such as ampicillin, aztreonam, cefotaxime, cefoxitin, ceftazidime, cefuroxime, cephalothin, ampicillin/sulbactam, gentamicin, tobramycin, norfloxacin, levofloxacin, ciprofloxacin and nitrofurantoin demonstrating this emergence.

4 | DISCUSSION

This study was designed as a follow-up to the 2016 veterinary colistin restriction (SAVC, 2016) and the SANVAD (Van Vuuren et al., 2007) focusing on commensal *E. coli* as a measuring tool within the Gauteng province of South Africa. Based on the OIE's recommendation on AMR surveillance, the study made use of swab samples collected from dressed chicken carcasses and chicken droppings in transport crates at various poultry abattoirs. To measure the prevalence of ABR, *E. coli* strains (438) obtained over a 2-year period were subjected to a panel of 24 antibiotic compounds. This presented with the opportunity of determining changes in the frequency of ABR in an indicator species in order to guide policies formulation.

4.1 | Colistin resistance

None of the *E. coli* isolates were resistant to colistin nor possessed the *mcr-1* gene. Relating this to the legislations guiding colistin use, the former medication is registered as a schedule IV drug in South Africa only available via veterinary prescription for therapeutic purposes (Mendelson et al., 2018). Until 2016, it was being compounded by the local veterinary pharmacy for use in the poultry industry (Mendelson et al., 2018); however, the introduction of tighter regulations, which limits prescription to conditions where no effective alternative treatment exists, has made such prescription rare and could perhaps explain our observation (SAVC, 2016). The latter would thus indicate the value of regulatory intervention in preventing emergence/reducing resistance to critical drugs, which in this case was achieved within a 5-year period. The effect was also similar to that reported in other countries that chose to restrict the veterinary use of colistin. In the EU, where veterinary colistin has also been restricted for use in cases when no effective alternative treatment exists, which has resulted in significant reduction in colistin consumption by about 50% (European Medicines Agency [EMA], 2020), with concurrent reduction in resistance from 1.9% to 0.7% in broilers (European Food Safety Authority and European Centre for Disease Prevention and Control [EFSA, ECDC], 2018; EFSA, ECDC, 2021). Similar trends have also been reported in Sweden (Swedres-Svarm, 2018); Denmark (Høg et al., 2018); Portugal and Great Britain (Duggett et al., 2018; Fournier et al., 2020). Lastly, China which used to be the major global consumer of colistin also demonstrated a reduction in resistance following a restriction on the drug use (Xia et al., 2019; Wang et al., 2020; Zhang et al., 2022). In 2016 when this policy came to effect, an estimated 8000 t reduction in the volume of colistin used in China was expected (Walsh & Wu, 2016). At present, the impact of that policy change is reflected in China where the prevalence of colistin resistance has reduced both in humans (55.9% i.e. 14.3%–6.3%) and animals (72.4% (i.e. 18.1%–5%)–85% (i.e. 34%–5.1%)) (Wang et al., 2020; Xia et al., 2019).

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TABLE 2 Proportions of antibacterial resistance in *Escherichia coli* strains obtained from chicken carcasses and faeces in South African poultry abattoirs in the years 2019 and 2020.

Antibacterials		Carcasses samples						Dropping samples						Control		
		2019 ($N = 122$)			2020 ($N = 106$)			2019 ($N = 112$)			2020 ($N = 98$)			($N = 17$)		
Classes	Drug compounds	Proportions (%)	95% CI		Proportions (%)	95% CI		Proportions (%)	95% CI		Proportions (%)	95% CI		Proportions (%)	95% CI	
Aminoglycosides	Amikacin	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
	Gentamicin	9.8	5.2	16.6	4.7	1.5	10.7	3.6	1	8.9	1	0	5.6	0	0	19.5
	Tobramycin	7.4	3.4	13.5	4.7	1.5	10.7	2.7	0.6	7.6	1	0	5.6	0	0	19.5
β-Lactams	Ampicillin	32	23.8	41	37.7	28.5	47.7	39.3	30.2	49	36.7	27.2	47.1	11.8	1.5	36.4
	Aztreonam	4.9	1.8	10.4	21.7	14.3	30.8	4.5	1.5	10.1	18.4	11.3	27.5	0	0	19.5
	Cefotaxime	8.2	4	14.6	36.8	27.6	46.7	8.9	4.4	15.8	27.6	19	37.5	0	0	19.5
	Cefoxitin	1.6	0.2	5.8	20.8	13.5	29.7	1.8	0.2	6.3	14.3	8	22.8	0	0	19.5
	Ceftazidime	8.2	4	14.6	36.8	27.6	46.7	9.8	5	16.9	25.5	17.2	35.3	0	0	19.5
	Cefepime	4.1	1.3	9.3	19.8	12.7	28.7	4.5	1.5	10.1	13.3	7.3	21.6	0	0	19.5
	Cefuroxime	4.1	1.3	9.3	30.2	21.7	39.9	4.5	1.5	10.1	21.4	13.8	30.9	0	0	19.5
	Ertapenem	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
	Imipenem	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
	Meropenem	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
β-Lactam + β-lactamase inhibitors	Amp/Sul	4.1	1.3	9.3	8.5	4	15.5	8.9	4.4	15.8	5.1	1.7	11.5	0	0	19.5
	Amox/Clav	4.9	1.8	10.4	23.6	15.9	32.8	6.2	2.5	12.5	19.4	12.1	28.6	5.9	0.1	28.7
	Piper/Tazo	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
Quinolones	Ciprofloxacin	15.6	9.6	23.2	18.9	11.9	27.6	16.1	9.8	24.2	9.2	4.3	16.7	11.8	1.5	36.4
	Levofloxacin	6.6	2.9	12.5	10.4	5.3	17.8	8	3.7	14.7	2	0.2	7.2	0	0	19.5
	Nalidixic Acid	47.5	38.4	56.8	43.4	33.8	53.4	33	24.4	42.6	21.4	13.8	30.9	11.8	1.5	36.4
	Norfloxacin	13.1	7.7	20.4	11.3	6	18.9	14.3	8.4	22.2	3.1	0.6	8.7	11.8	1.5	36.4
Polymyxin	Colistin	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
Tetracycline	Tigecycline	0	0	3	0	0	3.4	0	0	3.2	0	0	3.7	0	0	19.5
	Nitrofurantoin	4.9	1.8	10.4	5.7	2.1	11.9	8	3.7	14.7	4.1	1.1	10.1	0	0	19.5
Potentiated sulphonamide	Trim/Sulf	33.6	25.3	42.7	22.6	15.1	31.8	30.4	22	39.8	30.6	21.7	40.7	11.8	1.5	36.4

Note: Data presented in plain cells were interpreted using EUCAST guidelines; highlighted cells were interpreted using CLSI guidelines.
Abbreviations: Amox/Clav, amoxycillin/clavulanate; Amp/Sul, ampicillin/sulbactam; Piper/Tazo, piperacillin/tazobactam; Trim/Sulf, trimethoprim/sulphamethoxazole.

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TABLE 3 Frequency table showing strain's minimum inhibitory concentration distribution over the sampling period.

			Minimum inhibitory concentration (μg/mL)										
Drug	**Source**	***N***	**≤0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**MIC_{90}**
Amikacin	Car_19	122							121	1[a]			16
	Car_20	106							106				16
	Fec_19	112							112				16
	Fec_20	98							97	1[a]			16
	Control	17							17				16
Gentamicin	Car_19	122					108	2	12[a]				8
	Car_20	106					100	1	5[a]				4
	Fec_19	112					108		4[a]				4
	Fec_20	98					97		1[a]				4
	Control	17					16	1					4
Tobramycin	Car_19	122					111	2	9[a]				4
	Car_20	106					100	1	5[a]				4
	Fec_19	112					107	2	3[a]				4
	Fec_20	98					97		1[a]				4
	Control	17					17						4
Ampicillin	Car_19	122						84		38[a]			>16
	Car_20	106						66		40[a]			>16
	Fec_19	112						66	3	43[a]			>16
	Fec_20	98						62		36[a]			>16
	Control	17						14	1	2[a]			>16
Aztreonam	Car_19	122			112		5		5[a]				1
	Car_20	106			68		20	2	16[a]				>8
	Fec_19	112			102		7		3[a]				1
	Fec_20	98			71		13	6	8[a]				8
	Control	17			17								1
Cefotaxime	Car_19	122			113			5		4[a]			1
	Car_20	106			67			17	2	20[a]			>16
	Fec_19	112			102	1		5		4[a]			1
	Fec_20	98			70	1		14		13[a]			>16
	Control	17			17								1
Cefoxitin*	Car_19	122						115	5	2[a]			8
	Car_20	106						81	3	22[a]			>16
	Fec_19	112						102	8	2[a]			8
	Fec_20	98						79	5	14[a]			>16
	Control	17						17					8
Ceftazidime	Car_19	122			115		1	4	1	1			1
	Car_20	106			77		3	11	7	8			16
	Fec_19	112			104			6	1	1			1
	Fec_20	98			76		4	6	8	4			16
	Control	17			17								1

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TABLE 3 (Continued)

Drug	Source	N	Minimum inhibitory concentration (μg/mL)										MIC_{90}
			≤0.25	0.5	1	2	4	8	16	32	64	128	
Cefuroxime	Car_19	122						113	5	4[a]			8
	Car_20	106						67	7	32[a]			>16
	Fec_19	112						99	9	4[a]			16
	Fec_20	98						70	7	21[a]			>16
	Control	17						17					8
Cefepime	Car_19	122			118				4[a]				1
	Car_20	106			86		1	1	18[a]				>8
	Fec_19	112			108				4[a]				1
	Fec_20	98			85			1	12[a]				>8
	Control	17			17								1
Cephalothin	Car_19	122						76	32	14[a]			>16
	Car_20	106						44	21	41[a]			>16
	Fec_19	112						55	40	17[a]			>16
	Fec_20	98						42	28	28[a]			>16
	Control	17						13	1	3[a]			>16
Ertapenem	Car_19	122		122									0.5
	Car_20	106		106									0.5
	Fec_19	112		112									0.5
	Fec_20	98		98									0.5
	Control	17		17									0.5
Imipenem	Car_19	122			122								1
	Car_20	106			106								1
	Fec_19	112			112								1
	Fec_20	98			98								1
	Control	17			17								1
Meropenem	Car_19	122			122								1
	Car_20	106			106								1
	Fec_19	112			112								1
	Fec_20	98			98								1
	Control	17			17								1
Ampicillin/Sulbactam	Car_19	122						96	21	5[a]			16
	Car_20	106						68	29	9[a]			16
	Fec_19	112						79	23	10[a]			16
	Fec_20	98						66	27	5[a]			16
	Control	17						15	2				16
Amoxicillin/Clavulanate	Car_19	122						115	1	6[a]			8
	Car_20	106						81		25[a]			>16
	Fec_19	112						104	1	7[a]			8
	Fec_20	98						79		19[a]			>16
	Control	17						16		1[a]			8

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TABLE 3 (Continued)

Drug	Source	N	Minimum inhibitory concentration (μg/mL)										MIC_{90}
			≤0.25	0.5	1	2	4	8	16	32	64	128	
Piperacillin/Tazobactam	Car_19	122							122				16
	Car_20	106							106				16
	Fec_19	112							112				16
	Fec_20	98							98				16
	Control	17							17				16
Ceftazidime/Clavulanate	Car_19	122	116			1	5						0.25
	Car_20	106	82				24						4
	Fec_19	112	100			6	6						2
	Fec_20	98	77			4	17						4
	Control	17	17										0.25
Cefotaxime/Clavulanate	Car_19	122		116			6						0.5
	Car_20	106		82			21	3[a]					4
	Fec_19	112		105			7						0.5
	Fec_20	98		79			18	1[a]					4
	Control	17		17									0.5
Ciprofloxacin*	Car_19	122			96	7	19[a]						>2
	Car_20	106			84	2	20[a]						>2
	Fec_19	112			86	8	18[a]						>2
	Fec_20	98			86	3	9[a]						2
	Control	17			15		2[a]						>2
Levofloxacin*	Car_19	122				106	8	8[a]					4
	Car_20	106				88	7	11[a]					4
	Fec_19	112				98	5	9[a]					4
	Fec_20	98				90	6	2[a]					2
	Control	17				15	2						4
Nalidixic acid*	Car_19	122							64	58[a]			>16
	Car_20	106							60	46[a]			>16
	Fec_19	112							75	37[a]			>16
	Fec_20	98							77	21[a]			>16
	Control	17							15	2[a]			>16
Norfloxacin*	Car_19	122					97	9	16[a]				>8
	Car_20	106					93	1	12[a]				>8
	Fec_19	112					89	7	16[a]				>8
	Fec_20	98					92	3	3[a]				4
	Control	17					15		2[a]				>8
Colistin	Car_19	122				122							2
	Car_20	106				106							2
	Fec_19	112				112							2
	Fec_20	98				98							2
	Control	17				17							2

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TABLE 3 (Continued)

Drug	Source	N	Minimum inhibitory concentration (μg/mL)										MIC_{90}
			≤0.25	0.5	1	2	4	8	16	32	64	128	
Fosfomycin	Car_19	122									105	17[a]	>64
	Car_20	106									81	25[a]	>64
	Fec_19	112									97	15[a]	>64
	Fec_20	98									82	16[a]	>64
	Control	17									17		64
Tigecycline	Car_19	122			122								1
	Car_20	106			106								1
	Fec_19	112			112								1
	Fec_20	98			98								1
	Control	17			17								1
Nitrofurantoin	Car_19	122								96	20	6[a]	64
	Car_20	106								84	16	6[a]	64
	Fec_19	112								72	31	9[a]	64
	Fec_20	98								82	12	4[a]	64
	Control	17								17			32
			≤0.25	0.5	1	≤2/38	>2/38						
Trimethoprim/Sulphamethoxazole	Car_19	122				81	41[a]						>2
	Car_20	106				82	24[a]						>2
	Fec_19	112				78	34[a]						>2
	Fec_20	98				68	30[a]						>2
	Control	17				15	2[a]						>2

Note: Thick vertical lines indicate break points used; highlighted cells reflect drug concentrations tested; Car_19 = 2019 carcass samples; Car_20 = 2020 carcass samples; Fec_19 = 2019 faecal samples; Fec_2020 = 2020 faecal samples. Data presented were interpreted using EUCAST guidelines; however, cells with superscript (*) were interpreted using CLSI guidelines. Data presented on fosfomycin were generated using the broth microdilution technique.
[a]Minimum inhibitory concentration is ≥.

An important lesson learnt from the failed South Africa's initial attempt to ban veterinary colistin was the significance of an 'integrated and holistic multisectoral one health approach in combating AMR' (Mendelson et al., 2018). This first attempt did not receive the desired industry wide acceptance, as it relied largely on epidemiological data not locally obtained and was driven by isolated sectoral efforts. These inadequacies became more apparent when several critical stakeholders later became incorporated in evaluating the situation locally and culminated in prioritizing colistin use for critical cases (Mendelson et al., 2018). A key component to this is the later involvement of the SAVC which eventually directed all veterinary professionals to apply caution in using the said drug, a directive which effectively arrested veterinary colistin use in food animals in South Africa (SAVC, 2016). This latter approach with broader stakeholders inclusion is more in line with international best practices and prevented a dangerous precedent where veterinary drugs are banned without a proper local risk assessment (Collignon & Mcewen, 2019; WHO, FAO, OIE WHO, FAO, and OIE, 2015). AMR is a complex ecological phenomenon involving human, animal and environmental health and would require expertise from several sectors to tackle (Collignon & Mcewen, 2019; WHO, FAO, OIE WHO, FAO, and OIE, 2015; White & Hughes, 2019).

4.2 | General resistance

Looking at the phenotypic resistance data, one would quickly note a contrasting difference to the SANVAD report of 2007 (Van Vuuren et al., 2007). However, both reports present ABR data of commensal *E. coli* recovered from healthy chicken carcasses. Our data, however, tend to suggest that the prevalence of ABR to certain classes of drugs has declined. These reductions in proportions of resistance were noted towards quinolones and sulphonamides classes of antibacterials enrofloxacin (65.2%), nalidixic acid (63%) and sulphamethoxazole (87%) in SANVAD vs. ciprofloxacin (9.2%–18.9%), levofloxacin (2%–10.4%), norfloxacin (3.1%–14.3%), nalidixic acid (21.4%–47.5%) and sulphamethoxazole/trimethoprim (22.6%–33.6%) obtained in the present study. This is despite the very minimal/lack of changes in AMU legislation in South Africa as the SANVAD report was published

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and could perhaps be attributed to reduced drug use. Although there are no adequate consumption data on antimicrobials, the limited available information tends to support the latter claims. Eagar et al. (2012) demonstrated that veterinary antimicrobial consumption was considerably higher prior to the SANVAD report as compared to the 2015 data published by the NDoH (SANDH 2018). For example, it showed that veterinary consumption of sulphonamide/trimethoprim and penicillins had contracted by more than 50% since 2004.

On ampicillin resistance, the present study demonstrated a moderately higher proportion (32%–39.3%) than the SANVAD report of 2007 (28.3%), in spite of the more than 11-fold reduction in veterinary penicillins consumption per kilogram of meat produced since 2004 (Eagar et al., 2012; SANDH, 2018). Similar higher proportion (48.1%) was recently demonstrated by (McIver 2020) in the KwaZulu-Natal province of South Africa where they showed a remarkable increase in ampicillin resistance during week 2 of chicken fattening through to the bird slaughtering stage. These higher resistance proportions in much recent data than the SANVAD are surprising and contrary to report from Europe as one would expect much lower frequency of resistance as available national data show a reduction in drug use (Ceccarelli et al., 2020; Eagar et al., 2012; SANDH, 2018). Perhaps, our observations could be attributed to differences in the study design and testing methodologies adopted as MIC determination and isolate identification were automated in the present study contrary to the SANVAD which undertook these manually. It may also be that the poultry industry's specific need for penicillins has recently increased, and that the resistance data could just be a reflection of such recent surge. This is a plausible scenario as (Maruve & Essack, 2022) recently showed that 79.4% ($N = 102$) of South African veterinarians in a survey reported prescribing penicillins 'very often to always' during practice, with 77.5% ($N = 102$) regarding amoxicillin their first choice of antibacterial among others.

The aminoglycosides represent a negligible fraction of total veterinary antimicrobial drugs consumed in South Africa. In 2004, a low 0.07% was reported, a situation that has not changed significantly over the years, 0.08% in 2015. It was thus not surprising that the proportion of gentamicin resistance has remained low, that is 6.5% in the SANVAD report and 1%–9.8% in the present report. Similar low proportions of resistance were also reported in Germany during 2014 (7.0%) and 2016 (6.8%) in chicken (Mesa-Varona et al., 2020).

When data obtained during 2019 and 2020 were compared, the proportion of resistance seemed to be stable except for differences noted with the cephalosporins. The year 2020 saw a significantly higher proportion of resistance which could not be explained as farm AMU-specific information was not available. This observation warrants further investigations in the future; however, we hypothesize that (a) birds sampled in the earlier year may have originated from different farms to those sampled in 2020 as abattoirs sampled are commercial enterprises open to farmers who need to slaughter their livestock, (b) it may also be that the specific poultry farms where these birds originated from went through a period of increased need for the cephalosporins in the preceding year which could be responsible for this observation. This is plausible as more than 75% of strains demonstrating this phenotypic resistance originated from two specific abattoirs; however, there are no available data on consumption in this regard to support this speculation.

Similarly, in order to monitor changes in ABR frequency over time, the present data set was compared with earlier research data obtained during 2016 and 2018 by this group (Hassan et al., 2021, 2023). Interestingly, the proportions of ABR appeared to have remained fairly stable since 2016 with phenotypic resistance to quinolones, aminoglycosides and sulphamethoxazole–trimethoprim largely remaining unchanged for the period in question (Table S1). Additionally, there was no resistance recorded for carbapenems, tigecycline and piperacillin-tazobactam throughout the period. However, resistances to the cephalosporins, ampicillin, β-lactamase-inhibitor-potentiated penicillins and gentamicin were considerably higher during 2018, before declining in the preceding years. However, it is important to note that strains obtained during 2019/2020 originated from broiler chickens as opposed to those analysed in the preceding year, which were largely sourced from breeder and layer hens. Moreover, we know that as a management practise laying, chickens are usually kept for a much longer period than broilers (i.e. 70 vs. 5–6 weeks) and are more likely to have been exposed to antimicrobial drugs especially during recurrent disease episodes or failed therapy. More so, that strains obtained during 2016 and 2018 were isolated from sick birds and perhaps could have contributed to this higher level of resistance as strains originating from sick animals have higher propensity to demonstrate resistance to common drugs than the non-pathogenic strains (Gambi et al., 2022; OIE, 2015; Johnson et al., 2012).

In general, the distribution of MICs in the present study portrays a positive outlook with strains largely falling within the wild population category. More than 80% of the strains have not acquired phenotypic resistance to the fluoroquinolones and aminoglycosides. Acquired resistances (i.e. proportions of <50%) were more readily seen with the β-lactams (i.e. ampicillin), quinolones (i.e. nalidixic acid) and potentiated sulphonamide. Notably, no strain grew at the lowest tested concentration for carbapenems, tigecycline and piperacillin/tazobactam as these drugs are not licensed for use in food animals. As the country charts a way forward towards reducing veterinary consumption of antimicrobials, and while bearing in mind the peculiarities of the South African Livestock industry, the Republic will need to adopt best practices from other developed nations especially as considerable overlap exists between shared classes of antimicrobials in the animal and human health sector (Eagar et al., 2012; Nel et al., 2004; SANDH, 2018) with potential for shared AMR mechanism. This should include a commitment to reduce livestock disease burden through improved livestock management practice so as to reduce the need for veterinary AMU.

An important question that arises from this study would be to speculate on the impact of further regulatory restriction on the use of other antimicrobials like growth promoters. First to understand the control processes in South Africa, two distinct Acts have always regulated the veterinary use of drugs in South Africa, the earlier stock remedies Act (Act no. 36 of 1947), administered by the Department of Agriculture; and the much later Act 101 of 1965 (administered by the Department of Health) which was amended to accommodate/cater

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for veterinary medications not covered by the former (Naidoo & Eagar, 2019; Schellack et al., 2017). Although both Acts attempt to allow for optimum/rational use, some grey areas exist as certain antimicrobials are by law readily available over the counter to farmers without veterinary prescription (Naidoo & Eagar, 2019; Schellack et al., 2017). These antimicrobials which include, among others, growth promoters, antimastitis, anticoccidials and anthelminthics (i.e. sulphonamides, tetracyclines, macrolides, ionophores and streptogramins) are regulated by the stock remedies Act (Eagar & Naidoo, 2017; Naidoo & Eagar, 2019; Schellack et al., 2017). This is in contrast to countries like Sweden and Denmark where better controls are in place. In Sweden, a long standing ban (i.e. 35 years) on the use of antibiotics for growth promotion with a routine monitoring of antibiotic consumption linked to treatment guidelines and compulsory prescription requirement has resulted in reduced veterinary consumption by more than 70% to 10 t in 2018, with more than 90% of such prescription meant for individual animal therapy rather than for herd (Grundin et al., 2020; Ryan, 2019; Swedres-Svarm, 2018). Similarly, Denmark has in addition a unique 'Yellow Card Initiative' in place where veterinary antibiotic consumption thresholds are fixed to enforce reduction in antibiotic use (Høg et al., 2018; Ryan, 2019). In 2018, they reported a 51% reduction from 1994, with total volume consumed estimated at 100 t (Høg et al., 2018). These of course are linked to reduced livestock pathogen load through better livestock management practices.

If South Africa is to progress with its control on AMR, this will require amending the stock remedies Act (Act no 36 of 1947) to bring in tighter regulations on the veterinary use of antimicrobial agents. In doing this, the dual registration systems in place for veterinary drugs need to be harmonized into a single Act in order to avoid the duplication of effort. The Act should set threshold/establish realistic targets on volumes of veterinary antimicrobials to be consumed following extensive industry wide consultation without impacting on animal health and welfare. Ideally, this should increase veterinarians' oversight and mandate the withdrawal of all OTC dispensing of veterinary antimicrobials. This would go a long way in contributing to the desired reduction of AMR.

4.3 | Limitations

Premises sampled in this study were not randomly assigned as several declined and denied access to their facilities. Similarly, COVID-19-related regulations interfered with access to some premises; thus, our result may have been impacted in this regard reflecting only sampled premises and caution need to be exercised when interpreting the data. It is important to note that gaining access to these premises is usually difficult. It is a complex situation involving researchers, regulators and the livestock industry. To overcome this, we need to learn to work together and align our interest for the common good. The livestock industry is wary of researchers because of what they might likely discover and report, ultimately attracting regulator's attention which could affect their businesses. We, as researchers, need to instil our confidence in farmers and abattoir owners alike. We need to get them involved and enlightened on the benefits of research. They need to know that the essence of research is to improve yield and safer food.

The inability of the present study to demonstrate *mcr*-1 gene could be misleading as not all *E. coli* isolates were tested. More so *mcr-1* gene could be harboured unexpressed. To improve the recovery of these strains, we could modify the isolation technique by supplementing media with colistin sulphate to allow the growth of only resistant strains. However, the downside to this is that we would not be able to estimate the true prevalence of resistance. And of course, we may still not recover any strain if the prevalence of resistance is NIL. In addition, we would be adding to selective pressure exerted by colistin.

The fact that interpretations of data in this study were undertaken using the EUCAST and CLSI guidelines creates a scenario where resistance phenotypes are either underestimated or overestimated as BPs differ for certain drug compounds. This became necessary because panels used for analysis had in some cases drug concentration ranges not adapted for EUCAST interpretation. In these cases, the CLSI guidelines were used which in itself is a limitation.

5 | CONCLUSION

The findings of this study suggest that veterinary colistin restrictions have slowed *mcr-1* gene spread/propagation among commensal chicken *E. coli* and/or prevented it emergence/identification in sampled poultry abattoirs.

Prevalence of ABR in commensal chicken *E. coli* has generally declined in comparison to the SANVAD (2007). An observation could perhaps be attributed to overall reduction in veterinary antimicrobial consumption. Importantly, this data would also serve as a reference point for future studies.

AUTHOR CONTRIBUTIONS

Conceptualization; methodology; writing – review and editing; supervision; funding acquisition; resources: Vinny Naidoo. *Methodology; investigation; formal analysis; writing – original draft; review and editing; project administration*: Ibrahim Z. Hassan. *Methodology; formal analysis; writing – review and editing; supervision*: Daniel N. Qekwana.

ACKNOWLEDGEMENTS

We are extremely grateful to the South African Medical Research Council (SIR Grant-Risk assessment of the use of colistin in poultry medicine on human health in South Africa), the University of Pretoria and the South African National Research Foundation (I.Z.H. PhD grant) for funding this research.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial or non-financial interest to disclose.

FUNDING INFORMATION

South African Medical Research Council; the University of Pretoria; the South African National Research Foundation

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ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1315.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hassan, I. Z., Qekwana, D. N., & Naidoo, V. (2023). Prevalence of colistin resistance and antibacterial resistance in commensal *Escherichia coli* from chickens: An assessment of the impact of regulatory intervention in South Africa. *Veterinary Medicine and Science*, 1–14. https://doi.org/10.1002/vms3.1315

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