FPD-2023-0047-ver9-Hassan_1P
Type: research-article

FOODBORNE PATHOGENS AND DISEASE
Volume XX, Number XX, 2023

DOI: 10.1089/fpd.2023.0047

Original Article

Open camera or QR reader and scan code to access this article and other resources online.

Do Pathogenic *Escherichia coli* Isolated from *Gallus gallus* in South Africa Carry Co-Resistance Toward Colistin and Carbapenem Antimicrobials?

Ibrahim Zubairu Hassan, Daniel N. Qekwana, and Vinny Naidoo

Abstract

Colistin and carbapenems are critically important antimicrobials often used as a last resort to manage multidrug-resistant bacterial infections in humans. With limited alternatives, resistance to these antimicrobials is of concern as organisms could potentially spread horizontally rendering treatments ineffective. The aim of this study was to investigate co-resistance to colistin and carbapenems among *Escherichia coli* isolated from poultry in South Africa. Forty-six *E. coli* strains obtained from clinical cases of breeder and broiler chickens were used. In addition to other antibiotics, all the isolates were tested against colistin and carbapenems using broth microdilution. Multiplex polymerase chain reactions were used to investigate the presence of colistin (*mcr*-1 to 5) and carbapenem ($bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, and bla_{VIM}) resistance genes. Isolates exhibiting colistin resistance (>2 μg/mL) underwent a whole-genome sequencing analysis. Resistance to colistin (10.9%) and cefepime (6.5%) was noted with all colistin-resistant strains harboring the *mcr-1* gene. None of the *E. coli* isolates were resistant to carbapenems nor carried the other resistant genes (*mcr*-2 to 5, $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, and bla_{VIM}). The *mcr*-1-positive strains belonged to sequence types ST117 and ST156 and carried virulence genes *ompA*, *aslA*, *fdeC*, *fimH*, *iroN*, *iutA*, *tsh*, *pic*, *ast A* and *set 1A/1B*. In conclusion, clinical *E. coli* strains from chickens in this study possessed mobile resistance genes for colistin and several other clinically relevant antimicrobials but not carbapenems. Additionally, they belonged to sequence types in addition to carrying virulence factors often associated with human extraintestinal pathogenic *E. coli* infections. Thus, the potential risk of transmitting these strains to humans cannot be underestimated especially if sick birds are dispatched into the thriving poorly regulated Cornish hen industry. The need for routine veterinary surveillance and monitoring of antimicrobial resistance, AMU and the importance of strengthening regulations guiding the informal poultry sector remains important.

Keywords: colistin, carbapenem, *mcr-1*, *E. coli*, chicken, AMR

Introduction

Antimicrobial resistance is a major concern with both veterinary and human bacterial agents not only being multidrug resistant but also increasingly becoming extreme drug resistant (Durdu et al., 2019; Klima et al., 2014; Lubbers and Hanzlicek, 2013; Park et al., 2009; Sweeney et al., 2018; Walther et al., 2017). This concern has been reported in Gram-negative superbugs, such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *and Acinetobacter baumannii*, which are not only major human pathogens but are also responsive to few antimicrobials, notably colistin and carbapenems (Cassir et al., 2014; Parchem et al., 2016; Park et al., 2009). With such limited therapeutic selection, resistance to these remaining drugs is of concern (Durdu et al., 2019). While resistance in people would likely arise from the direct use of antimicrobials, the zoonotic potential of organisms and their role in transmissible resistance needs to be considered especially from foodborne microbial contamination.

Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa.

For colistin resistance, concern lies with mobilized colistin resistance (*mcr*), which has emerged as a global concern in both human and animal health sector (Liu et al., 2016; Luo et al., 2020; Wang et al., 2020a; Xavier et al., 2016; Yin et al., AU5 ▶ 2017). Since their first description by Liu et al. (2015), 10 different *mcr* coding genes have been identified (Li et al., 2020; Luo et al., 2020; Sun et al., 2018; Wang et al., 2020a).

The overuse of colistin in poultry, pig, and calves has been singled out as a major driver for *mcr* gene selection and resistance development (Liu et al., 2016; Luo et al., 2020; Sun et al., 2018; Trung et al., 2017). Of concern is that the *mcr* gene is transferable within and between bacterial species via plasmid conjugation conferring resistance to an otherwise known susceptible strain (Liu et al., 2016). Thus, with the relatively high incidence of these *mcr* genes among veterinary organisms, it raises the question on the subsequent threat on human health (Zhang et al., 2016).

Another concern is the increasing incidence of carbapenemase-producing *Enterobacteriaceae* from animal and animal products (Abdallah et al., 2015; Al Bayssari et al., 2015; Fischer et al., 2012; Köck et al., 2018). While it is not fully understood how this occurs as carbapenems use is highly limited in animals due to costs (Madec et al., 2017), the implications of carbapenemase-producing *Enterobacteriaceae* in animals are far reaching due to the potential for zoonotic transmission. In humans, carbapenemases render most penicillins, cephalosporins, and carbapenems ineffective (Doi and Paterson, 2015; Zmarlicka et al., 2015). Moreover, strains carrying genes encoding the carbapenemases have been shown to spread and persist (Abraham et al., 2016; Fischer et al., 2017) and have tendencies of transferring resistance genes to other microorganisms (Castanheira et al., 2013).

Of greater concern than the veterinary organisms carrying resistance to either carbapenems or colistin alone has been the co-occurrence of *mcr-1* and carbapenemase-encoding genes (bla_{NDM}) in *E. coli* strains in chicken meat, chicken ceca, cloaca swabs, and/or feces (Liu et al., 2017; Wang et al., 2017b; Yao et al., 2016). While not properly characterize, the co-expression of resistance genes has in part been attributed to environmental contamination from dogs, flies, and wild birds (Wang et al., 2017b), highlighting the need for an holistic approach in tackling antimicrobial resistance (AMR).

With both colistin and carbapenems resistance being individually noted in several apparently susceptible strains (Apostolakos and Piccirillo, 2018; Fattouh et al., 2016; Hassan et al., 2021; Karlowsky et al., 2017; Lentz et al., 2016), a scenario of silent co-resistance gene transfer to humans and/or animals is a concern. The aim of this study was to investigate co-resistance to colistin and carbapenems among *E. coli* isolated from poultry in South Africa, the virulence genes carried and their potential risk on human health. Furthermore, resistance to several other clinically relevant antibacterial agents was evaluated.

Materials and Methods

Study population and sampling

Ethical approval for this study was obtained from the University of Pretoria animal ethics committee (V098-17). The study used biobanked *E. coli* strains ($n = 46$) isolated by Deltamune (Pty) Ltd bacteriology laboratory in 2018 from unhealthy domestic chicken (*Gallus gallus*) in Pretoria, South Africa. Of the isolates, 40 (87%) originated from breeder hens, whereas the rest (13%, 6/46) were obtained from broiler birds. Samples were obtained during postmortem evaluation at multiple farm locations from suspected cases of colibacillosis. Additional information on specific tissues sampled during postmortem is not accessible; however, these are typically aseptically collected swab samples from presenting lesions.

Isolation of E. coli

Samples were processed within 36 h of collection using standard bacteriological methods, as previously published (Theobald et al., 2019). In brief, *E. coli* were isolated on 5% Oxoid blood tryptose agar and Oxoid MacConkey agar without salt and crystal violet (Thermo Fisher Scientific, Hampshire, United Kingdom). Plates were incubated aerobically for up to 24 h at 37°C. Single red non-mucoid presumptive *E. coli* colonies per sample were subjected to biochemical test using Kovack's reagent (Merck, Darmstadt, Germany), and oxidase reagent (Merck) with Selecta-media Hugh–Leifson tubes (Thermo Fisher Scientific).

Antibacterial susceptibility testing

Susceptibility was determined using the WalkAway 40 plus MicroScan machine (Beckman Coulter, Inc., CA) as per instructions. Antibiogram was obtained automatically after 18–24 h of bacterial growth against selected agents (Table 2). For quality control, *E. coli* ATCC 25922 and a known colistin-resistant strain were included in the analysis. All interpretations were carried out using the EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute) guidelines of 2019 and 2018, respectively (CLSI, 2018; EUCAST, 2019).

Multiplex polymerase chain reaction assay

All isolates were subjected to a multiplex polymerase chain reaction (PCR) to detect the presence of *mcr-1* to 5 genes associated with colistin resistance, and the $bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, and bla_{VIM} genes associated with carbapenem resistance. The gene primer sequences used were as described by previous researchers (Borowiak et al., 2017; Gonçalves et al., 2017; Liu et al., 2016; Poirel et al., 2011; Rebelo et al., 2018; Samuelsen et al., 2011).

Colistin multiplex reaction. MCR-1-positive strain and a *mcr-5* DNA available at the University of Pretoria were used as positive controls. For every *mcr* PCR conducted, 12.5 μL of DreamTaq Green 2 × Master Mix (Thermo Fisher Scientific, Waltham, MA), 6.5 μL of nuclease-free water, 0.5 μL of each primer solution (10 μM), and a microliter of DNA lysate were used. The thermocycling condition was maintained at 94°C for 15 min +25 × (94°C for 30 s + 58°C for 90 s + 72°C for 60 s) +72°C for 10 min, using a MiniAmp Plus Thermal Cycler (Thermo Fisher Scientific) (Cavaco et al., 2016).

Carbapenem multiplex reaction. For every *bla* PCR conducted, 12.5 μL of 2 × MyTaq HS Master Mix (Bioline, London, United Kingdom), 8.5 μL of nuclease-free water, 0.5 μL of each primer solution (10 μM), and a microliter of DNA lysate were used. Positive and negative controls were included for all

runs. The thermocycling condition was maintained at 95°C for 2 min +35 × (95°C for 30 s + 57°C for 40 s + 72°C for 60 s) +72°C for 3 min. Resulting amplicons were subjected to agarose gel electrophoresis, as described previously.

Whole-genome sequencing

Colistin-resistant isolates were subjected to whole-genome sequencing using an Illumina MiSeq Technology (Illumina, Inc., San Diego, CA), as previously described (Trung et al., 2017). Nucleotide sequences were blasted through the ResFinder, PlasmidFinder, and VFDB (Virulence Factor Database) databases to identify antibacterial resistance genes, plasmids, and virulence factors, respectively, within the strains (Carattoli et al., 2014; Chen et al., 2016; Chen et al., 2005; Zankari et al., 2012). Multilocus sequence typing was also undertaken to characterize strains into sequence types (ST) using the putative seven housekeeping genes, i.e., adk, fumC, gyrB, icd, mdh, purA, and recA (Paul et al., 2013; Wirth et al., 2006).

Data management and analysis

The antibacterial resistance phenotypes of strains were coded in SPSS (IBM SPSS Statistics for Windows, Version 26.0; IBM Corp, Armonk, NY) with proportions of ABR and multiple drug resistance (MDR) determined. The MIC_{90} of the various drug compounds tested were extrapolated directly from the MicroScan machine.

Results

Antibacterial susceptibility

Following antibiogram analysis, almost all (97.8%, 45/46) strains were resistant to at least one antibacterial drug. *E. coli* were resistance to tetracycline (82.2%) and the beta lactams (ampicillin [63%], ampicillin/sulbactam [60.9%], and piperacillin [57.8%]). Significantly, 10.9% of isolates were resistance to colistin and 6.5% to the fourth-generation cephalosporin and cefepime. No carbapenem resistance was detectable (Table 1). Multiple drug resistance (MDR) was noted in 84.8% (39/46, 95% confidence interval 71.1–93.7) of the *E. coli* isolates, with two strains being phenotypically classified as ESBL producers by the MicroScan.

Multiplex PCR and gel electrophoresis

Following multiplex PCR, five strains demonstrated the presence of *mcr-1* gene (Fig. 1). These strains represent all colistin-resistant isolates obtained from this study. None of the strains carried *mcr-2* to 5; bla_{OXA-48}, bla_{NDM}, and bla_{VIM} genes.

Table 1. Antibiotic Resistance Proportion of Clinical Avian *Escherichia coli* Isolates Obtained from South Africa in 2018

Drug compounds	*Estimate (%)*	*95% CI Lower–upper*	*MIC_{90}*	*BP*
Amikacin	2.2 (1/45)	0.1–11.5	8	>16
Ampicillin	63.0 (29/46)	47.5–76.8	>16	>8
Aztreonam	10.9 (5/46)	3.6–23.6	8	>4
Cefotaxime	47.8 (22/46)	32.9–63.1	8	>2
Cefoxitin[a]	39.1 (18/46)	25.1–54.6	>16	≥32
Ceftazidime	41.3 (19/46)	27.0–56.8	16	>4
Cefuroxime	47.8 (22/46)	32.9–63.1	>16	>8
Chloramphenicol	11.1 (5/45)	3.7–24.1	>16	>8
Ciprofloxacin[a]	15.2 (7/46)	6.3–28.9	>2	≥4
Colistin	10.9 (5/46)	3.6–23.6	>4	>2
Doripenem[a]	0.0 (0/45)	0.0–07.9	1	≥4
Ertapenem	0.0 (0/46)	0.0–07.7	0.5	>0.5
Gentamicin	37.8 (17/45)	23.2–52.5	>8	>4
Imipenem	0.0 (0/46)	0.0–07.7	1	>4
Levofloxacin[a]	8.7 (4/46)	02.4–20.8	4	≥8
Meropenem	0.0 (0/46)	0.0–07.7	1	>8
Minocycline[a]	20.0 (9/45)	9.6–34.6	>8	≥16
Nalidixic acid[a]	45.7 (21/46)	30.9–61.0	>16	≥32
Nitrofurantoin	2.2 (1/46)	0.1–11.5	32	>64
Norfloxacin	33.3 (15/45)	19.5–48.0	>1	>1
Piperacillin	57.8 (26/45)	42.2–72.3	>64	>16
Piperacillin/tazobactam	0.0 (0/45)	0.0–07.7	8	>16/4
Tetracycline[a]	82.2 (37/45)	67.9–92.0	>8	≥16
Tigecycline[a]	0.0 (0/46)	0.0–07.7	1	>1
Trimethoprim/sulfamethoxazole	30.4 (14/46)	17.7–45.8	>4	>4/76
Tobramycin	6.7 (3/45)	1.4–17.9	4	>4
Cefepime	6.5 (3/46)	1.4–17.9	1	>4
Ampicillin/sulbactam	60.9 (28/46)	45.4–74.9	16	>8/4
Amoxicillin/clavulanate	43.5 (20/46)	28.9–58.9	>16	>8/2

Values in parenthesis (*n*/*N*).
[a]CLSI and EUCAST breakpoints were used for interpretation.
BP, break point; CI, confidence interval; CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC_{90}, minimum inhibitory concentration required to kill 90% of the bacterial population.

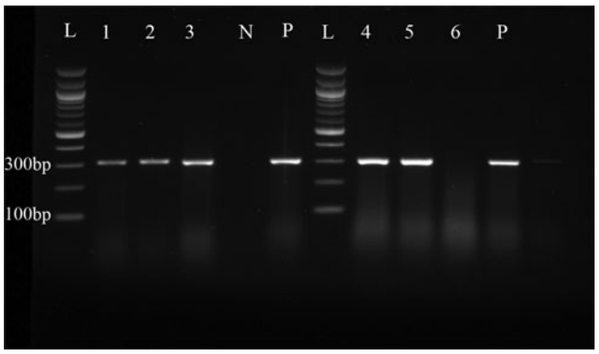

FIG. 1. Gel image of APEC strains carrying the *mcr-1* gene (309 bp) following PCR and electrophoresis. APEC, avian pathogenic *Escherichia coli*; L, DNA step ladder; N, negative control; numerals 1–5, positive strains; 6, negative strain; P, positive control; PCR, polymerase chain reaction.

Whole-genome sequencing

Sequencing confirmed the presence of the *mcr-1* gene, together with several other resistance genes and plasmids (Table 2), and were in agreement with the phenotypic resistance displayed.

All five strains carried virulence genes coding for the flagella (*flgGH*, *fliIMPG*, *flhA*), enterobactin (*entDFSCEBA*, *fepACGDB*, *fes*), type I fimbriae (*fimBEHGFDCIA*), type III secretion system (*espXRLY*), curli (*csgBDEFG*), adhesin (*fdeC*), outer membrane protein (*ompA*), and chemotaxis regulatory protein (*cheY*). In addition, some strains possessed genes coding for heat stable enterotoxin (*astA*), putative arylsulfatase (*aslA*), toxin subunit (set 1A/B), serine protease precursor (pic), temperature-sensitive hemagglutinin (*tsh*), aerobactin, salmochelin receptor, and the shu locus protein.

Discussion

Despite having a very organized poultry industry, South Africa also has a thriving informal poultry sector where trade of live birds is poorly regulated (Abolnik, 2007). A common practice is the trade of spent breeder and layer hens from commercial farms to the informal township markets where they are slaughtered and dressed in the open (Abolnik, 2007; Fourie, 1995). With some local consumer's preference for this so-called ''Cornish'' or ''Rocks'' hens as source of poultry meat and about 25 million birds in production at any given time (Abolnik, 2017), the potential risk of an antibacterial resistant strain being transmitted to a human cannot be underestimated. More so that human contact with some of these live birds could potentially pose a risk.

Carbapenem and colistin resistance

Unlike studies in China which demonstrated chicken *E. coli* lineage co-harboring *mcr-1* and carbapenemase resistance genes (Lin et al., 2020; Liu and Song, 2019; Yang

Table 2. Resistome and Plasmid Content of Avian Pathogenic *Escherichia coli* Strains Isolated from Chicken

ID	*Phenotypic resistance*	*Resistance genes*	*Plasmids*	*Sequence types*
1	Ampicillin, chloramphenicol, colistin, tetracycline, sulfamethoxazole	tet(A), blaTEM-1B, mcr-1.1, mdf(A), tet(34), aac(3)-Ib, aadA2, cmlA1, ant(3″)-Ia, sul3, mef(B), tet(M)	IncFII, ColRNAI, IncFIA(HI1), Col(MG828), IncHI1B(R27), IncHI1A, p0111, IncFIB(AP001918)	7329
2	Colistin, norfloxacin, tetracycline, trimethoprim, sulfamethoxazole, minocycline[i]	mcr-1.1, ant(3″)-Ia, dfrA14, mdf(A), tet(34)_1, tet(M), qnrS1, tet(A)	IncFIA(HI1), IncHI1B(R27), IncHI1A, IncX1, p0111	155
3	Ampicillin, chloramphenicol, ciprofloxacin, colistin, levofloxacin, norfloxacin, minocycline, nalidixic acid, nitrofurantoin, piperacillin, tetracycline, trimethoprim, sulfamethoxazole, ampicillin/sulbactam	mcr-1.1, aph(6)-Id, aph(3″)-Ib, sul2, oqxB, oqxA, tet(B), ant(3″)-Ia, dfrA14, tet(34), aph(3′)-Ia, blaTEM-1B, mdf(A)	Col156, ColpVC, Col(MG828), Col(MG828), Col(MG828), IncI2, p0111, IncI1, IncFIA, IncFIB(AP001918), IncFIC(FII), IncFII	156
4	Colistin, nalidixic acid, tetracycline, trimethoprim, sulfamethoxazole, minocycline[i], norfloxacin[i]	tet(A), sul3, mcr-1.1, mdf(A), aph(3″)-Ib, aph(6)-Id, ant(3″)-Ia, dfrA12, aadA2, sul1	Col(MG828), IncI2, IncFIB(AP001918), IncHI2A, IncFIC(FII), IncFII, IncFIC(FII), IncFII, IncHI2, TrfA	117
5	Ampicillin, chloramphenicol, colistin, minocycline, piperacillin, tetracycline, trimethoprim, sulfamethoxazole, ampicillin/sulbactam, tobramycin[i]	catA1, ant(3″)-Ia, dfrA17, tet(B), oqxA, oqxB, sul2, aph(3″)-Ib, aph(6)-Id, mcr-1.1, blaTEM-1B, aph(3′)-Ia, mdf(A), tet(34)	IncFIB(AP001918), IncQ1, Col156, IncFIC(FII), IncFII, IncI2	5764

Except for gentamicin, resistance phenotype associated with the specific aminoglycoside resistance gene identified was not tested for.

et al., 2016), none of the *E. coli* isolates in this study demonstrated phenotypic nor genetic resistance toward carbapenems. This absence of carbapenem resistance supports information that the carbapenems are not used for production due to cost. Also, it was important to demonstrate the absence of resistance since migratory birds and stray dogs have been implicated in the transmission of resistance (Wang et al., 2017b). While the co-expression of colistin and carbapenem resistance was not identified, five colistin-resistant strains that carried the *mcr-1* gene were noted.

This level of colistin resistance was similar to 13.6% (108/797) reported by Perreten et al. (2016). The observation was somewhat unexpected as South Africa has a restriction on use of colistin in the food-producing industry, a policy that came to effect in 2016. Countries that have implemented a similar restriction such as China, Portugal, and Great Britain have shown a decline in prevalence of *mcr*-1-associated colistin resistance in <5 years (Duggett et al., 2018; Fournier et al., 2020; Theobald et al., 2019; Wang et al., 2020b). While more recent data have now shown a decline in South Africa (3.61%) (NDoH, 2022), the present high colistin resistance could be indicative of continued colistin use despite the current veterinary restriction or due to colistin-resistant organism circulating in the farms.

Virulence factors and sequence types

AU10► WGS analysis in the *mcr*-1-containing strains ($n=5$) revealed that these strains possessed several virulence genes (*ompA*, *aslA*, *fdeC*, *csgBDEFG, fimH*, *iroN*, *iutA*, *iucDCBA*, *chuVUYWT*, *shuXAS*), known to play key roles in bacterial serum resistance, invasion of brain microvascular endothelial cells, adhesion, biofilm formation, iron acquisition, persistence, and evasion of host defense mechanisms (Burkhard and Wilks, 2007; Chairatana et al., 2015; Connell et al., 1996; de Lorenzo et al., 1986; Easton et al., 2014; Furrer et al., 2002; Gophna et al., 2001; Hoffman et al., 2000; Kim, 2001; Krishnan and Prasadarao, 2012; Müller et al., 2009; Nesta et al., 2012; Prasadarao et al., 1996; Prigent-Combaret et al., 2000; Saldaña et al., 2009; Shulman et al., 2018; Suits et al., 2009; Thompson et al., 1999; Tong and Guo, 2009; Vizcarra et al., 2016; Warner et al., 1981).

More importantly, some of these strains carried genes (*tsh*, *aslA*, *pic*) similar to those reported in human extraintestinal pathogenic *E. coli* and could potentially have zoonotic implications (Ewers et al., 2007; Habouria et al., 2019; Moulin-Schouleur et al., 2006; Navarro-Garcia and Elias, 2011; Ronco et al., 2017). These genes play vital roles in hemolysis and invasion of the brain microvascular endothelial cells.

The ST117 strain stood out as it carried additional genes coding for toxins (*ast A*, *set 1A*/*1B*) and hemolysin (*tsh*). This AU11► APEC lineage has frequently been associated with avian colibacillosis (Cummins et al., 2019; Ronco et al., 2017) and is being viewed as an emerging human pathogen globally associated with blood and urinary tract infections (Cummins et al., 2019; Macesic et al., 2017; Manges and Johnson, 2012; Mora et al., 2012; Quan et al., 2017; Vincent et al., 2010; Wang et al., 2017a). Indeed, this sequence type has been isolated from chicken meat meant for human consumption in the Netherlands (Kluytmans-Van Den Bergh et al., 2016).

Similarly, another *E. coli* sequence type (i.e., ST156) often associated with human infections was identified in the present study (Bilal et al., 2021; He et al., 2017; Lin et al., 2020; Rashid et al., 2015). This sequence type has been isolated from fresh vegetables, human blood stream infections, and avian fecal swab in China (Lin et al., 2020; Liu and Song, 2019; Yang et al., 2016). Additionally, *E. coli* ST155 associated with human blood stream and urinary tract infections in Germany and Uganda, respectively, was also identified in the present study (Decano et al., 2021; Neumann et al., 2020). Two other rare sequence types (i.e., ST5764 and ST7329) were identified from a broiler and breeder hen, respectively.

Resistance to other antibacterials

Although the focus of this study was mainly on colistin and carbapenem resistance, a high proportion (82.2%) of isolates were resistant to tetracycline, with strains recruited for WGS possessing several *tet* genes (*tet A*, *tet B*, *tet M*, and *tet 34*). This may be due to the tetracyclines being readily available for over-the-counter use (Eagar and Naidoo, 2017; Mendelson et al., 2018) and being the second most consumed class of veterinary antimicrobials in SA, including the use for growth ◄AU12 promotion (SANDH, 2018).

Furthermore, they are widely used for treatment of bacterial infections, mycoplasma, chlamydial, and rickettsia avian diseases (Chopra and Roberts, 2001). Similar high proportions of tetracycline resistance have also been reported in other countries, including China, Nigeria, Egypt, Zimbabwe, and Senegal (Ahmed et al., 2013; Jiang et al., 2011; Liu et al., 2017; Olarinmoye et al., 2013; Saidi et al., 2012; Vounba et al., 2018). In contrast, in Europe where more effective control measures are in place, the prevalence's of resistance are much lower (Mesa-Varona et al., 2020; VMD, 2019). From this, it is likely that the over-the-counter availability of the tetracyclines in South Africa is a major contributor to the resistance seen.

The proportions of isolates resistant to the penicillin's and cephalosporin's were also high due to the blaTEM-1B gene, which codes for β-lactamase enzymes, and could have resulted from the common use of the cephalosporin's, particularly ceftiofur to prevent mortality in day-old chicks (Agunos et al., 2017; Dutil et al., 2010; Liu et al., 2017). This higher than expected cephalosporin resistance could present a problem for humans if transferred through the food chain (Agunos et al., 2017; Dutil et al., 2010; Vieira et al., 2011), and potentially necessitate an increase use for last line defense drugs such as carbapenems and/or colistin (Gauzit et al., 2015; Nordmann and Cornaglia, 2012; Wilson, 2017).

Limitations

The origin of isolates used in this study is limited to one laboratory source; therefore, the results cannot be extrapolated to entire poultry population in the country due to variations in drug use patterns. In addition, only strains resistant to colistin were subjected to whole-genome sequencing. However, the latter does not preclude any meaningful conclusion drawn from strains lacking such analysis as our phenotypic data were consistent with the WGS results.

Conclusions

The *mcr-1* colistin gene is still present in *E. coli* isolates from the SA poultry industry, and it is a public health

concern. More so that some of these positive strains carry virulence genes similar to those associated with human diseases. The results seem to suggest that carbapenem resistance–associated genes and other colistin resistance determinants are uncommon among *E. coli* isolates in the poultry sector. Of concern is the high prevalence of resistance to the tetracycline and β-lactam classes of antibiotics, which could potentially present a problem if transferred through the food chain to humans. The outcome highlights the need for routine veterinary surveillance and monitoring of AMR to curb the spread. In addition, regulations guiding veterinary use of antimicrobials and practices in the poultry informal sector must be strengthened.

Acknowledgment

We appreciate the kind gesture of Prof. Jaap Wagenaar of Utrecht University, The Netherlands, for undertaken the whole-genome sequencing analysis.

Authors' Contributions

V.N.: Conceptualization, methodology, writing—review and editing, supervision, funding acquisition, and resources. I.Z.H.: Methodology, investigation, formal analysis, writing—original draft, review and editing, and project administration. D.N.Q.: Methodology, formal analysis, writing—review and editing, and supervision.

Disclosure Statement

No competing financial interests exist.

AU13 ▶ ### Funding Information

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 study.

References

Abdallah H, Reuland E, Wintermans B, et al. Extended-spectrum β-lactamases and/or carbapenemases-producing Enterobacteriaceae isolated from retail chicken meat in Zagazig, Egypt. PLoS One 2015;10(8):e0136052; doi: 10.1371/journal.pone.0136052

AU14 ▶ Abolnik C. Molecular Epidemiology of Newcastle Disease and Avian Influenza in South Africa. University of Pretoria; 2007.

Abolnik C. History of Newcastle Disease in South Africa. Onderstepoort J Veterinary Res 2017;84(1):1–7; doi: 10.4102/ojvr.v84i1.1306

Abraham S, O'Dea M, Trott DJ, et al. Isolation and plasmid characterization of carbapenemase (IMP-4) producing Salmonella enterica Typhimurium from cats. Sci Rep 2016;6:35527; doi: 10.1038/srep35527

Agunos A, Léger DF, Carson CA, et al. Antimicrobial use surveillance in broiler chicken flocks in Canada, 2013–2015. PLoS One 2017;12(6):e0179384; doi: 10.1371/journal.pone.0179384

Ahmed AM, Shimamoto T, Shimamoto T. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int J Med Microbiol 2013;303(8):475–483; doi: 10.1016/j.ijmm.2013.06.009

Al Bayssari C, Olaitan AO, Dabboussi F, et al. Emergence of OXA-48-producing *Escherichia coli* clone ST38 in fowl. Antimicrob Agents Chemother 2015;59(1):745–746; doi: 10.1128/AAC.03552-14

Apostolakos I, Piccirillo A. A review on the current situation and challenges of colistin resistance in poultry production. Avian Pathol 2018;47(6):546–558; doi: 10.1080/03079457.2018.1524573

Bilal H, Rehman TU, Khan MA, et al. Molecular epidemiology of mcr-1, blaKPC-2, and blaNDM-1 harboring clinically isolated *Escherichia coli* from Pakistan. Infect Drug Resist 2021;14:1467; doi: 10.2147/IDR.S302687

Blackburn D, Husband A, Saldaña Z, et al. Distribution of the ◀ AU15 *Escherichia coli* common pilus among diverse strains of human enterotoxigenic *E. coli*. J Clin Microbiol 2009;47(6):1781–1784; doi: 10.1128/jcm.00260-09

Borowiak M, Fischer J, Hammerl JA, et al. Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. enterica serovar Paratyphi B. J Antimicrob Chemother 2017;72(12):3317–3324; doi: 10.1093/jac/dkx327

Burkhard KA, Wilks A. Characterization of the outer membrane receptor ShuA from the heme uptake system of Shigella dysenteriae substrate specificity and identification of the heme protein ligands. J Biol Chem 2007;282(20):15126–15136; doi: 10.1074/jbc.M611121200

Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014;58(7):3895–3903; doi: 10.1128/aac.02412-14

Cassir N, Rolain J-M,Brouqui P. A new strategy to fight antimicrobial resistance: The revival of old antibiotics. Front Microbiol 2014;5:551; doi: 10.3389/fmicb.2014.00551

Castanheira M, Deshpande LM, Farrell SE, et al. Update on the prevalence and genetic characterization of NDM-1-producing Enterobacteriaceae in Indian hospitals during 2010. Diagn Microbiol Infect Dis 2013;75(2):210–213; doi: 10.1016/j.diagmicrobio.2012.10.017

Cavaco L, Mordhorst H, Hendriksen R. PCR for Plasmid-Mediated Colistin Resistance Genes: mcr-1 and mcr-2 (Multiplex). Protocol optimized at National Food Institute: Denmark; 2016.

Chairatana P, Zheng T, Nolan EM. Targeting virulence: Salmochelin modification tunes the antibacterial activity spectrum of β-lactams for pathogen-selective killing of *Escherichia coli*. Chem Sci 2015;6(8):4458–4471; doi: 10.1039/C5SC00962F

Chen L, Yang J, Yu J, et al. VFDB: A reference database for bacterial virulence factors. Nucleic Acids Res 2005;33(suppl_1):D325–D328; doi: 10.1093/nar/gki008

Chen L, Zheng D, Liu B, et al. VFDB 2016: Hierarchical and refined dataset for big data analysis—10years on. Nucleic Acids Res 2016;44(D1):D694–D697; doi: 10.1093/nar/gkv1239.

Chopra I, Roberts M. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 2001;65(2):232–260; doi: 10.1128/mmbr.65.2.232-260.2001

CLSI. Performance Standards for Antimicrobial Susceptibility ◀ AU16 Testing. 28th ed. CLSI Supplement M100. 2018.

Coburn B, Sekirov I, Finlay BB. Type III secretion systems and disease. Clin Microbiol Rev 2007;20(4):535–549; doi: 10.1128/cmr.00013-07

Connell I, Agace W, Klemm P, et al. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. Proc Natl Acad Sci U S A 1996;93(18):9827–9832; doi: 10.1073/pnas.93.18.9827

Cummins ML, Reid CJ, Chowdhury PR, et al. Whole genome sequence analysis of Australian avian pathogenic *Escherichia coli* that carry the class 1 integrase gene. Microb Genom 2019;5(2); doi: 10.1099/mgen.0.000250

de Lorenzo V, Bindereif A, Paw B, et al. Aerobactin biosynthesis and transport genes of plasmid ColV-K30 in *Escherichia coli* K-12. J Bacteriol 1986;165(2):570–578; doi: 10.1128/jb.165.2.570-578.1986

Decano AG, Pettigrew K, Sabiiti W, et al. Pan-resistome characterization of uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* strains circulating in Uganda and Kenya, isolated from 2017–2018. Antibiotics 2021;10(12):1547; doi: 10.3390/antibiotics10121547

Doi Y, Paterson DL. Carbapenemase-producing enterobacteriaceae. In: Seminars in Respiratory and Critical Care Medicine. NIH Public Access; 2015; p. 74; doi: 10.1055/s-0035-1544208

Duggett NA, Randall LP, Horton RA, et al. Molecular epidemiology of isolates with multiple mcr plasmids from a pig farm in Great Britain: The effects of colistin withdrawal in the short and long term. J Antimicrob Chemother 2018;73(11):3025–3033; doi: 10.1093/jac/dky292

Durdu B, Koc MM, Hakyemez IN, et al. Risk factors affecting patterns of antibiotic resistance and treatment efficacy in extreme drug resistance in intensive care unit-acquired *Klebsiella pneumoniae* infections: A 5-year analysis. Med Sci Monit 2019;25:174; doi: 10.12659/MSM.911338

Dutil L, Irwin R, Finley R, et al. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis 2010;16(1):48; doi: 10.3201/eid1601.090729

Eagar H, Naidoo V. Veterinary antimicrobial stewardship in South Africa. Int Biol Rev 2017;1(2); doi: 10.18103/ibr.v1i2.1367

Easton DM, Allsopp LP, Phan M-D, et al. The intimin-like protein FdeC is regulated by H-NS and temperature in enterohemorrhagic *Escherichia coli*. Appl Environ Microbiol 2014;80(23):7337–7347; doi: 10.1128/AEM.02114-14

Erume J, Berberov EM, Kachman SD, et al. Comparison of the contributions of heat-labile enterotoxin and heat-stable enterotoxin b to the virulence of enterotoxigenic *Escherichia coli* in F4ac receptor-positive young pigs. Infect Immun 2008;76(7):3141–3149; doi: 10.1128/iai.01743-07

EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoints tables for interpretation of MIC's and zone diameters version 9.0, 2019. 2019.

Ewers C, Li G, Wilking H, et al. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: How closely related are they? Int J Med Microbiol 2007;297(3):163–176; doi: 10.1016/j.ijmm.2007.01.003

Fattouh R, Tijet N, McGeer A, et al. What is the appropriate meropenem MIC for screening of carbapenemase-producing Enterobacteriaceae in low-prevalence settings? Antimicrob Agents Chemother 2016;60(3):1556–1559; doi: 10.1128/aac.02304-15

Figueira R, Holden DW. Functions of the Salmonella pathogenicity island 2 (SPI-2) type III secretion system effectors. Microbiology 2012;158(5):1147–1161; doi: 10.1099/mic.0.058115-0

Fischer J, Rodríguez I, Schmoger S, et al. *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. J Antimicrob Chemother 2012;67(7):1793–1795; doi: 10.1093/jac/dks108

Fischer J, San José M, Roschanski N, et al. Spread and persistence of VIM-1 carbapenemase-producing Enterobacteriaceae in three German swine farms in 2011 and 2012. Vet Microbiol 2017;200:118–123; doi: 10.1016/j.vetmic.2016.04.026

Fourie P. THE SOUTH AFRICAN POULTRY INDUSTRY/Die Suid-Afrikaanse Pluimveebedryf. Agrekon 1995;34(1):29–36; doi: 10.1080/03031853.1995.9524289

Fournier C, Aires-de-Sousa M, Nordmann P, et al. Occurrence of CTX-M-15-and MCR-1-producing Enterobacterales in pigs in Portugal: Evidence of direct links with antibiotic selective pressure. Int J Antimicrob Agents 2020;55(2):105802; doi: 10.1016/j.ijantimicag.2019.09.006

Furrer JL, Sanders DN, Hook-Barnard IG, et al. Export of the siderophore enterobactin in *Escherichia coli*: Involvement of a 43kDa membrane exporter. Mol Microbiol 2002;44(5):1225–1234; doi: 10.1046/j.1365-2958.2002.02885.x

Garnett JA, Martínez-Santos VI, Saldaña Z, et al. Structural insights into the biogenesis and biofilm formation by the *Escherichia coli* common pilus. Proc Natl Acad Sci U S A 2012;109(10):3950–3955; doi: 10.1073/pnas.1106733109

Gauzit R, Pean Y, Alfandari S, et al. Carbapenem use in French hospitals: A nationwide survey at the patient level. Int J Antimicrob Agents 2015;46(6):707–712; doi: 10.1016/j.ijantimicag.2015.08.013

Gonçalves GB, Furlan JPR, Vespero EC, et al. Spread of multidrug-resistant high-risk *Klebsiella pneumoniae* clones in a tertiary hospital from southern Brazil. Infection, Genet Evol 2017;56:1–7; doi: 10.1016/j.meegid.2017.10.011

Gophna U, Barlev M, Seijffers R, et al. Curli fibers mediate internalization of *Escherichia coli* by eukaryotic cells. Infect Immun 2001;69(4):2659–2665; doi: 10.1128/iai.69.4.2659-2665.2001

Habouria H, Pokharel P, Maris S, et al. Three new serine-protease autotransporters of Enterobacteriaceae (SPATEs) from extra-intestinal pathogenic *Escherichia coli* and combined role of SPATEs for cytotoxicity and colonization of the mouse kidney. Virulence 2019;10(1):568–587; doi: 10.1080/21505594.2019.1624102

Hassan I, Wandrag B, Gouws J, et al. Antimicrobial resistance and mcr-1 gene in *Escherichia coli* isolated from poultry samples submitted to a bacteriology laboratory in South Africa, Vet World 2021;14(10):2662–2669; doi: 10.14202/vetworld.2021.2662-2669

He Q-w, Xu X-h, Lan F-j, et al. Molecular characteristic of mcr-1 producing *Escherichia coli* in a Chinese university hospital. Ann Clin Microbiol Antimicrob 2017;16(1):1–5; doi: 10.1186/s12941-017-0207-z.

Hoffman JA, Badger JL, Zhang Y, et al. *Escherichia coli* K1 aslAContributes to invasion of brain microvascular endothelial cells in vitro and in vivo. Infect Immun 2000;68(9):5062–5067; doi: 10.1128/iai.68.9.5062-5067.2000

Jiang H-X, Lü D-H, Chen Z-L, et al. High prevalence and widespread distribution of multi-resistant *Escherichia coli* isolates in pigs and poultry in China. Vet J 2011;187(1):99–103; doi: 10.1016/j.tvjl.2009.10.017

Karlowsky JA, Lob SH, Kazmierczak KM, et al. In vitro activity of imipenem against carbapenemase-positive En-

terobacteriaceae isolates collected by the SMART global surveillance program from 2008 to 2014. J Clin Microbiol 2017;55(6):1638–1649; doi: 10.1128/jcm.02316-16

Kim KS. Escherichia coli translocation at the blood-brain barrier. Infect Immun 2001;69(9):5217–5222; doi: 10.1128/iai.69.9.5217-5222.2001

Klima CL, Zaheer R, Cook SR, et al. Pathogens of bovine respiratory disease in North American feedlots conferring multidrug resistance via integrative conjugative elements. J Clin Microbiol 2014;52(2):438–448; doi: 10.1128/jcm.02485-13

Kluytmans–Van Den Bergh MF, Huizinga P, Bonten MJ, et al. Presence of mcr-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. Eurosurveillance 2016;21(9):30149; doi: 10.2807/1560-7917.ES.2016.21.9.30149

Köck R, Daniels-Haardt I, Becker K, et al. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: A systematic review. Clin Microbiol Infect 2018;24(12):1241–1250; doi: 10.1016/j.cmi.2018.04.004

Krishnan S, Prasadarao NV. Outer membrane protein A and OprF: Versatile roles in Gram-negative bacterial infections. FEBS J 2012;279(6):919–931; doi: 10.1111/j.1742-4658.2012.08482.x

Lentz SA, de Lima-Morales D, Cuppertino VM, et al. Letter to the editor: *Escherichia coli* harbouring mcr-1 gene isolated from poultry not exposed to polymyxins in Brazil. Eurosurveillance 2016;21(26):30267; doi: 10.2807/1560-7917.ES.2016.21.26.30267

Lerouge I, Vanderleyden J. O-antigen structural variation: Mechanisms and possible roles in animal/plant–microbe interactions. FEMS Microbiol Rev 2002;26(1):17–47; doi: 10.1111/j.1574-6976.2002.tb00597.x

Li Y, Dai X, Zeng J, et al. characterization of the global distribution and diversified plasmid reservoirs of the colistin resistance gene mcr-9. Sci Rep 2020;10(1):1–10; doi: 10.1038/s41598-020-65106-w

Lin Y, Yang L, Lu L, et al. Genomic features of an *Escherichia coli* ST156 strain harboring chromosome-located mcr-1 and plasmid-mediated blaNDM-5. Infect Genet Evol 2020;85:104499; doi: 10.1016/j.meegid.2020.104499

Liu B-T, Song F-J. Emergence of two *Escherichia coli* strains co-harboring mcr-1 and blaNDM in fresh vegetables from China. Infect Drug Resist 2019;12:2627; doi: 10.2147/idr.s211746

Liu B-T, Song F-J, Zou M, et al. High incidence of *Escherichia coli* strains coharboring mcr-1 and blaNDM from chickens. Antimicrob Agents Chemother 2017;61(3); doi: 10.1128/aac.01204-16

Liu Y-Y, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis 2016;16(2):161–168; doi: 10.1016/S1473-3099(15)00424-7

Lubbers BV, Hanzlicek GA. Antimicrobial multidrug resistance and coresistance patterns of Mannheimia haemolytica isolated from bovine respiratory disease cases—A three-year (2009–2011) retrospective analysis. J Vet Diagn Investig 2013;25(3):413–417; doi: 10.1177/1040638713485227

Luo Q, Wang Y, Xiao Y. Prevalence and transmission of mobilized colistin resistance (mcr) gene in bacteria common to animals and humans. Biosafety Health 2020; doi: 10.1016/j.bsheal.2020.05.001

Macesic N, Green D, Wang Z, et al. Detection of mcr-1-carrying Escherichia coli causing bloodstream infection in a New York City hospital: Avian origins, human concerns? In: Open Forum Infectious Diseases. Oxford University Press: 2017; doi: 10.1093/ofid/ofx115

Madec J-Y, Haenni M, Nordmann P, et al. Extended-spectrum β-lactamase/AmpC-and carbapenemase-producing Enterobacteriaceae in animals: A threat for humans? Clin Microbiol Infect 2017;23(11):826–833; doi: 10.1016/j.cmi.2017.01.013

Manges AR, Johnson JR. Food-borne origins of *Escherichia coli* causing extraintestinal infections. Clin Infect Dis 2012;55(5):712–719; doi: 10.1093/cid/cis502

Mendelson M, Brink A, Gouws J, et al. The One Health stewardship of colistin as an antibiotic of last resort for human health in South Africa. Lancet Infect Dis 2018;18(9):e288–e294; doi: 10.1016/S1473-3099(18)30119-1

Mesa-Varona O, Kaspar H, Grobbel M, et al. Phenotypical antimicrobial resistance data of clinical and non-clinical *Escherichia coli* from poultry in Germany between 2014 and 2017. PLos One 2020;15(12):e0243772; doi: 10.1371/journal.pone.0243772

Mora A, López C, Herrera A, et al. Emerging avian pathogenic *Escherichia coli* strains belonging to clonal groups O111: H4-D-ST2085 and O111: H4-D-ST117 with high virulence-gene content and zoonotic potential. Vet Microbiol 2012;156(3–4):347–352; doi: 10.1016/j.vetmic.2011.10.033

Moulin-Schouleur M, Schouler C, Tailliez P, et al. Common virulence factors and genetic relationships between O18: K1: H7 *Escherichia coli* isolates of human and avian origin. J Clin Microbiol 2006;44(10):3484–3492; doi: 10.1128/jcm.00548-06

Müller SI, Valdebenito M, Hantke K. Salmochelin, the long-overlooked catecholate siderophore of Salmonella. Biometals 2009;22(4):691–695; doi: 10.1007/s10534-009-9217-4

Navarro-Garcia F, Elias WP. Autotransporters and virulence of enteroaggregative *E. coli*. Gut microbes 2011;2(1):13–24; doi: 10.4161/gmic.2.1.14933

NDoH. Surveillance for Antimicrobial Resistance and Consumption of Antimicrobials in South Africa, 2021. In: Health ed. Republic of South Africa; 2022. ◀AU17

Nesta B, Spraggon G, Alteri C, et al. FdeC, a novel broadly conserved *Escherichia coli* adhesin eliciting protection against urinary tract infections. MBio 2012;3(2); doi: 10.1128/mbio.00010-12

Neumann B, Rackwitz W, Hunfeld K-P, et al. Genome sequences of two clinical *Escherichia coli* isolates harboring the novel colistin-resistance gene variants mcr-1.26 and mcr-1.27. Gut Pathogens 2020;12(1):1–7; doi: 10.1186/s13099-020-00375-4

Nordmann P, Cornaglia G. Carbapenemase-producing Enterobacteriaceae: A call for action! Clin Microbiol Infect 2012;18(5):411–412; doi: 10.1111/j.1469-0691.2012.03795.x

Olarinmoye A, Oladele O, Adediji A, et al. Antibiograms of avian pathogenic *Escherichia coli* isolates from commercial layers with colibacillosis in Southwest Nigeria. Malays J Microbiol 2013;9(4):317–325.

Parchem NL, Bauer KA, Cook CH, et al. Colistin combination therapy improves microbiologic cure in critically ill patients with multi-drug resistant gram-negative pneumonia. Eur J Clin Microbiol Infect Dis 2016;35(9):1433; doi: 10.1007/s10096-016-2681-1

Park YK, Peck KR, Cheong HS, et al. Extreme drug resistance in *Acinetobacter baumannii* infections in intensive care units,

South Korea. Emerg Infect Dis 2009;15(8):1325; doi: 10.3201/eid1508.080772

Paul S, Linardopoulou EV, Billig M, et al. Role of homologous recombination in adaptive diversification of extraintestinal *Escherichia coli*. J Bacteriol 2013;195(2):231–242; doi: 10.1128/jb.01524-12

Perreten V, Strauss C, Collaud A, et al. Colistin resistance gene mcr-1 in avian pathogenic *Escherichia coli* in South Africa. Antimicrob Agents Chemother 2016; doi: 10.1128/AAC.00548-16

Poirel L, Walsh TR, Cuvillier V, et al. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70(1):119–123; doi: 10.1016/j.diagmicrobio.2010.12.002

Prasadarao NV, Wass CA, Weiser JN, et al. Outer membrane protein A of *Escherichia coli* contributes to invasion of brain microvascular endothelial cells. Infect Immun 1996;64(1):146–153; doi: 10.1128/iai.64.1.146-153.1996

Prigent-Combaret C, Prensier G, Le Thi TT, et al. Developmental pathway for biofilm formation in curli-producing *Escherichia coli* strains: Role of flagella, curli and colanic acid. Environ Microbiol 2000;2(4):450–464; doi: 10.1046/j.1462-2920.2000.00128.x

Quan J, Li X, Chen Y, et al. Prevalence of mcr-1 in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: A multicentre longitudinal study. Lancet Infect Dis 2017;17(4):400–410; doi: 10.1016/S1473-3099(16)30528-X

Rashid M, Rakib MM, Hasan B. Antimicrobial-resistant and ESBL-producing *Escherichia coli* in different ecological niches in Bangladesh. Infect Ecol Epidemiol 2015;5(1):26712; doi: 10.3402/iee.v5.26712

Rebelo AR, Bortolaia V, Kjeldgaard JS, et al. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Eurosurveillance 2018;23(6):17-00672; doi: 10.2807/1560-7917.ES.2018.23.6.17-00672

Ronco T, Stegger M, Olsen RH, et al. Spread of avian pathogenic *Escherichia coli* ST117O78: H4 in Nordic broiler production. BMC Genomics 2017;18(1):1–8; doi: 10.1186/s12864-016-3415-6.

Saidi B, Mafirakureva P, Mbanga J. Antimicrobial resistance of *Escherichia coli* isolated from chickens with colibacillosis in and around Harare, Zimbabwe. Avian Dis 2012;57(1):152–154; doi: 10.1637/10325-081512-Case.1

Saldaña Z, Xicohtencatl-Cortes J, Avelino F, et al. Synergistic role of curli and cellulose in cell adherence and biofilm formation of attaching and effacing *Escherichia coli* and identification of Fis as a negative regulator of curli. Environ Microbiol 2009;11(4):992–1006; doi: 10.1111/j.1462-2920.2008.01824.x

Samuelsen Ø, Thilesen CM, Heggelund L, et al. Identification of NDM-1-producing Enterobacteriaceae in Norway. J Antimicrob Chemother 2011;66(3):670–672; doi: 10.1093/jac/dkq483

AU18► SANDH. Surveillance for antimicrobial resistance and consumption of antibiotics in South Africa. 2018.

Sarkar MK, Paul K, Blair D. Chemotaxis signaling protein CheY binds to the rotor protein FliN to control the direction of flagellar rotation in *Escherichia coli*. Proc Natl Acad Sci U S A 2010;107(20):9370–9375; doi: 10.1073/pnas.1000935107

Shabana I. Detection of the enteroaggregative heat-stable enterotoxin 1 nucleotide sequences among diarrhegenic *Escherichia coli*. Austin J Clin Immunol 2014;1(2):1009.

Shulman A, Yair Y, Biran D, et al. The *Escherichia coli* type III secretion system 2 has a global effect on cell surface. Mbio 2018;9(4); doi: 10.1128/mbio.01070-18

Suits MD, Lang J, Pal GP, et al. Structure and heme binding properties of *Escherichia coli* O157: H7 ChuX. Protein Sci 2009;18(4):825–838; doi: 10.1002/pro.84

Sun J, Zhang H, Liu Y-H, et al. Towards understanding MCR-like colistin resistance. Trends Microbiol 2018; doi: 10.1016/j.tim.2018.02.006

Sweeney MT, Lubbers BV, Schwarz S, et al. Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens. J Antimicrob Chemother 2018;73(6):1460–1463; doi: 10.1093/jac/dky043

Terashima H, Kojima S, Homma M. Flagellar motility in bacteria: Structure and function of flagellar motor. Int Rev Cell Mol Biol 2008;270:39–85; doi: 10.1016/S1937-6448(08)01402-0

Thanassi DG, Hultgren SJ. Multiple pathways allow protein secretion across the bacterial outer membrane. Curr Opin Cell Biol 2000;12(4):420–430; doi: 10.1016/S0955-0674(00)00111-3

Theobald S, Etter EMC, Gerber D, et al. Antimicrobial resistance trends in *Escherichia coli* in South African Poultry: 2009–2015. Foodborne Pathog Dis 2019;16(9):652–660; doi: 10.1089/fpd.2018.2612

Thompson JM, Jones HA, Perry RD. Molecular characterization of the hemin uptake locus (hmu) from *Yersinia pestis* and analysis of hmu mutants for hemin and hemoprotein utilization. Infect Immun 1999;67(8):3879–3892; doi: 10.1128/iai.67.8.3879-3892.1999

Tong Y, Guo M. Bacterial heme-transport proteins and their heme-coordination modes. Arch Biochem Biophys 2009;481(1):1–15; doi: 10.1016/j.abb.2008.10.013

Trung NV, Matamoros S, Carrique-Mas JJ, et al. Zoonotic transmission of mcr-1 colistin resistance gene from small-scale poultry farms, Vietnam. Emerg Infect Dis 2017;23(3):529; doi: 10.3201/eid2303.161553

Ud-Din AIMS, Roujeinikova A. Methyl-accepting chemotaxis proteins: A core sensing element in prokaryotes and archaea. Cell Mol Life Sci 2017;74(18):3293–3303; doi: 10.1007/s00018-017-2514-0

Vieira AR, Collignon P, Aarestrup FM, et al. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: An ecological study. Foodborne Pathog Dis 2011;8(12):1295–1301; doi: 10.1089/fpd.2011.0950

Vincent C, Boerlin P, Daignault D, et al. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis 2010;16(1):88; doi: 10.3201/eid1601.091118

Vizcarra IA, Hosseini V, Kollmannsberger P, et al. How type 1 fimbriae help *Escherichia coli* to evade extracellular antibiotics. Sci Rep 2016;6(1):1–13; doi: 10.1038/srep18109

VMD. UK Veterinary Antibiotic Resistance and Sales Surveillance Report UK-VARSS 2018. Veterinary Medicines Directorate; 2019. ◄AU19

Vounba P, Kane Y, Ndiaye C, et al. Molecular characterization of *Escherichia coli* isolated from chickens with Colibacillosis in Senegal. Foodborne Pathog Dis 2018;15(8):517–525; doi: 10.1089/fpd.2017.2394

Walther B, Tedin K, Lübke-Becker A. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol 2017;200:71–78; doi: 10.1016/j.vetmic.2016.05.017

Wang C, Feng Y, Liu L, et al. Identification of novel mobile colistin resistance gene mcr-10. Emerg Microbes Infect 2020a;9(1):508–516; doi: 10.1080/22221751.2020.1732231

Wang Y, Tian G-B, Zhang R, et al. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive Enterobacteriaceae in patients and healthy adults from China: An epidemiological and clinical study. Lancet Infect Dis 2017a;17(4):390–399; doi: 10.1016/S1473-3099(16)30527-8

Wang Y, Xu C, Zhang R, et al. Changes in colistin resistance and mcr-1 abundance in Escherichia coli of animal and human origins following the ban of colistin-positive additives in China: An epidemiological comparative study. Lancet Infect Dis 2020b; doi: 10.1016/S1473-3099(20)30149-3

Wang Y, Zhang R, Li J, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. Nat Microbiol 2017b;2(4):1–7; doi: 10.1038/nmicrobiol.2016.260

Warner P, Williams P, Bindereif A, et al. ColV plasmid-specific aerobactin synthesis by invasive strains of *Escherichia coli*. Infect Immun 1981;33(2):540–545; doi: 10.1128/iai.33.2.540-545.1981

Weiser JN, Gotschlich EC. Outer membrane protein A (OmpA) contributes to serum resistance and pathogenicity of *Escherichia coli* K-1. Infect Immun 1991;59(7):2252–2258; doi: 10.1128/iai.59.7.2252-2258.1991

WHO. Critically Important Antimicrobials for Human Medicine. World Health Organization: Geneva; 2019.

Wilson APR. Sparing carbapenem usage. J Antimicrob Chemother 2017;72(9):2410–2417; doi: 10.1093/jac/dkx181

Wirth T, Falush D, Lan R, et al. Sex and virulence in *Escherichia coli*: An evolutionary perspective. Mol Microbiol 2006; 60(5):1136–1151; doi: 10.1111/j.1365-2958.2006.05172.x

Xavier BB, Lammens C, Ruhal R, et al. Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. Eurosurveillance 2016; 21(27):30280; doi: 10.2807/1560-7917.ES.2016.21.27.30280

Yang R-S, Feng Y, Lv X-Y, et al. Emergence of NDM-5-and MCR-1-producing *Escherichia coli* clones ST648 and ST156 from a single muscovy duck (*Cairina moschata*). Antimicrob Agents Chemother 2016;60(11):6899–6902; doi: 10.1128/aac.01365-16

Yao X, Doi Y, Zeng L, et al. Carbapenem-resistant and colistin-resistant *Escherichia coli* co-producing NDM-9 and MCR-1. Lancet Infect Dis 2016;16(3):288–289; doi: 10.1016/S1473-3099(16)00057-8

Yin W, Li H, Shen Y, et al. Novel plasmid-mediated colistin resistance gene mcr-3 in *Escherichia coli*. MBio 2017;8(3); doi: 10.1128/mbio.00543-17

Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67(11):2640–2644; doi: 10.1093/jac/dks261

Zhang X-F, Doi Y, Huang X, et al. Possible transmission of mcr-1-harboring *Escherichia coli* between companion animals and human. Emerg Infect Dis 2016;22(9):1679; doi: 10.3201/eid2209.160464

Zmarlicka MT, Nailor MD, Nicolau DP. Impact of the New Delhi metallo-beta-lactamase on beta-lactam antibiotics. Infect Drug Resist 2015;8:297; doi: 10.2147/idr.s39186

Address correspondence to:
Ibrahim Zubairu Hassan ◀AU20
Veterinary Pharmacology/Toxicology Section ◀AU21
Department of Paraclinical Sciences
Faculty of Veterinary Science
University of Pretoria
1-42.1
Onderstepoort 0110
South Africa

E-mail: aburo001@gmail.com

AUTHOR QUERY FOR FPD-2023-0047-VER9-HASSAN_1P

AU1: Please note that gene symbols in any article should be formatted as per the gene nomenclature. Thus, please make sure that gene symbols, if any in this article, are italicized.
AU2: Please identify (highlight or circle) all authors' surnames for accurate indexing citations.
AU3: Please include the Clinical Trial Registration number, if applicable, at the end of the abstract.
AU4: Please expand ''AMU.''
AU5: Please note that Ref. ''Liu et al. (2015)'' is cited in text but not provided in the reference list. Please provide the reference in list.
AU6: Please check the usage of ''OBJ'' in the sentence ''Colistin-resistant isolates were subjected …''.
AU7: Please define ''ABR.''
AU8: Please expand ''MIC90.''
AU9: Please expand ''ESBL.''
AU10: Please define ''WGS.''
AU11: Please expand ''APEC.''
AU12: Please define ''SA.''
AU13: Please provide the grant numbers for funding agency(ies)/institution(s).
AU14: In Ref. Abolnik (2007), please mention the location of the publisher.
AU15: References ''Blackburn et al. (2009), Coburn et al. (2007), Erume et al. (2008), Figueira et al. (2012), Garnett et al. (2012), Lerouge et al. (2002), Sarkar et al. (2010), Shabana et al. (2014), Terashima et al. (2008), Thanassi et al. (2000), Ud-Din et al. (2017), Weiser and Gotschlich (1991), and WHO (2019)'' are not cited in the text. Please insert the citations in the text.
AU16: In Ref. CLSI (2018), please mention the publisher's name and its location.
AU17: In Ref. NDoH (2022), please expand ''NDoH.'' Also mention the publisher's name.
AU18: In Ref. SANDH (2018), please provide the other publication details or URL.
AU19: In Ref. VMD (2019), please mention the location of the publisher.
AU20: Please mention the degree abbreviation (e.g., MS, MD, PhD) of the corresponding author.
AU21: ''Veterinary Pharmacology/Toxicology Section'' is mentioned in corresponding author's address, but not mentioned in author's affiliation. Please check and fix for consistency.