



Occurrence of Extended-Spectrum Beta-Lactamase and Quinolone Resistance Genes among *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Poultry, Domestic Pigs and Environ in Msimbazi River Basin in Tanzania

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ABSTRACT: We investigated the occurrence and distribution of genes encoding for extended-spectrum beta-lactamase (ESBL) production and quinolone resistance among multi-drug resistant (MDR) *Escherichia coli* and *Klebsiella pneumoniae* isolated from poultry, domestic pigs, and environment samples in the Msimbazi basin in Tanzania. A total of 130 non-duplicated isolates obtained from the poultry (n = 40), domestic pigs (n = 52) and environment (n = 38) were screened for ESBL genes (*bla*_{CTX-M}, *bla*_{TEM}, and *bla*_{SHV}) and plasmid-mediated quinolone resistance (PMQR) genes (*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, and *aac* (6)-*lb-cr*) using polymerase chain reaction (PCR). The most commonly detected ESBL genes were *bla*_{CTX-M} (31.5%, n=41) and *bla*_{TEM} (10%, n=13), while quinolone resistance genes were *qnrS* (27.7%, n=36), *qnrB* (6.9%, n=9) and *aac* (6)-*lb-cr* (5.4%, n=7). ESBL gene *bla*_{SHV} and quinolone resistance genes *qnrA*, *qnrC*, and *qepA* were not detected in any of the isolates. Resistance genes were highest in isolates from the environmental samples (86%, n=33), followed by poultry 72.5% (n=29), and domestic pigs 21.4%, (n=9). Overall, the most frequent resistance gene was *qnrS*, followed by *bla*_{CTX-M} and *bla*_{TEM}. About 17.7% (n=23) of the *E. coli* and *K. pneumoniae* isolates depicted co-existence of ESBL and quinolone resistance genes. One Health interventional measures, involving different disciplines across human, animal, and environmental sectors, are needed to control the spread of antimicrobial resistance in the Msimbazi River Basin in Tanzania.

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In Tanzania, the demand for short-cycled food animals such as poultry and domestic pigs has increased in recent years (Frumence *et al.*, 2021; Mdegela *et al.*, 2021). For example, the consumption of poultry was estimated to rise by 258% from 130,000 tons in 2017 to 465,600 tons in 2020, and that of domestic pigs by

69% from 22,000 in 2017 to 37,200 tons in 2022, respectively (Michael *et al.*, 2018). This high demand has led to intensive production methods, associated with poor animal husbandry practices, overcrowding, high disease frequency, and overstretched veterinary extension services (Mdegela *et al.*, 2021; Mshana *et*

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al., 2021). The antimicrobial usage in the animal sector in Tanzania from 2010 to 2017 amounted to 12,147,491 kilograms (Sangeda *et al.*, 2021). The most commonly used antimicrobial agents were tetracycline, sulphonamides and trimethoprim, quinolones, aminoglycosides, and beta-lactams (Mdegela *et al.*, 2021; Sangeda *et al.*, 2021). Tetracycline alone accounted for 66.6% (8,090,798 kg) of the total drugs (Sangeda *et al.*, 2021). Due to the imprudent use of veterinary drugs in animal farming, high levels of antimicrobial resistance bacteria, including multidrug-resistant strains, have been isolated from chickens, domestic pigs (Caudell *et al.*, 2017), and the surrounding environment (Mshana *et al.*, 2021).

The high level of antimicrobial use (AMU) and antimicrobial resistance (AMR) poses a threat to public and animal health given the fact that they are associated with the development and spread of antimicrobial resistance genes, which can be transferred across animal, human, and environment compartments as shown in a previous study (Minja *et al.*, 2021; Subbiah *et al.*, 2020). Indeed, conjugative plasmids have been responsible for disseminating *bla*_{CTX-M-15} among humans, animals, and the environment (Minja *et al.*, 2021). Additionally, the resistant genes *bla*_{CTX-M}, *qnrS*, and *aac(6)-lb-cr* were found to circulate between humans, domestic and companion animals, fish and environment (Subbiah *et al.*, 2020).

In East Africa, a range of genetic diversity of ESBL and carbapenem-resistant genes from *Escherichia coli* and *Klebsiella pneumoniae* isolates including *bla*_{TEM}, *bla*_{OXA-1}, *bla*_{CMY-2}, *bla*_{CTX-M}, *bla*_{SHV}, NDM, VIM, IMP, KPC, and *bla*_{OXA-48} from human, animals and the environment have been reported which were attributed to a range of sources including mutations, selection pressure and genes transfer (Katale *et al.*, 2020). In Tunisia, a study by (Grami *et al.*, 2016) identified IncH12-type plasmid spreading the *bla*_{CTX-M-1} and *mcr-I* in farm chick, which were reported in cattle in France and food samples in Portugal (Valiakos and Kapna, 2021). In Nigeria, the plasmid-mediated quinolone resistance (PMQR) from *Escherichia coli* strains was recovered from healthy pigs and poultry that resembled those of human origin (Fortini *et al.*, 2011). Furthermore, the spread of similar antimicrobial resistance genes from the community, human, animals, and environment have been reported in Tanzania, Cameroon, Nigeria, Angola, and Netherlands (Adesoji *et al.*, 2015; Dohmen *et al.*, 2015; Founou *et al.*, 2018; Moremi *et al.*, 2017; Ribeiro *et al.*, 2016). A review study reported that humans exposed to poultry farms carried carbapenem-resistant Enterobacteriaceae

(CRE) with close relation to the environmental isolates (Köck *et al.*, 2018).

Members of the Enterobacteriaceae family particularly *Escherichia coli* and *Klebsiella pneumoniae* that are resistant to both quinolones and beta-lactams occur worldwide (Founou *et al.*, 2018; Moremi *et al.*, 2017), causing widespread infections that are difficult to treat (Shaikh *et al.*, 2015). Molecular characterization of antimicrobial resistance genes offers a deeper insight into the possible mechanisms of transmission, an important source of information in the control of AMR spread (Alouache *et al.*, 2014; Liu *et al.*, 2018). Some of the most commonly involved resistance genes include *bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M} for extended-spectrum beta-lactamase (ESBL); VIM, KPC, NDM, OXA, and IMP for carbapenem and *qnrA*, *qnrB*, *qnrC*, *qnrD* and *qnrS* for quinolone resistance (Athanasakopoulou *et al.*, 2021; Dohmen *et al.*, 2015; Geser *et al.*, 2012; Salah *et al.*, 2019; Taggar *et al.*, 2020; Tshitshi *et al.*, 2020; Varela *et al.*, 2021). These genes are located on the plasmids and mobile genetic elements, which make their transmission extremely easy (Alouache *et al.*, 2014; Minja *et al.*, 2021; Ssekatawa *et al.*, 2018; Stadler *et al.*, 2018).

The Msimbazi river basin in Tanzania has a myriad of human activities making it a hot spot for the spread of AMR bacteria and resistant genes between human-animal-environment compartments (Kayombo and Mayo, 2018; Kahangwa, 2021; Mrutu *et al.*, 2013). Phenotypic studies conducted in the basin showed very high levels of ESBL-producing and quinolone resistant strains among bacteria isolated from poultry, domestic pigs, and the environment (Kimera, *et al.*, 2021a,b).

However, all these studies were limited to phenotypic screening of antibiotic resistant pathogens and none focused on the occurrence of resistant genes. Hence, the objective of this paper was to investigate the occurrence of extended-spectrum beta-lactamase (ESBL) and quinolone resistance genes among *Escherichia coli* and *Klebsiella pneumoniae* isolated from poultry, domestic pigs and environ in Msimbazi River Basin in Tanzania.

MATERIAL AND METHODS

Bacteria isolates: A total of 130 non-duplicate *Escherichia coli* (n=95) and *Klebsiella pneumoniae* (n=35) isolates from poultry (n=40), domestic pigs (n=52), and environment (n=38) were selected from stored isolates obtained between July 2020 and April 2021 (Kimera, *et al.*, 2021a,b). These isolates were randomly selected from a total of 504 isolates from domestic pigs (n=193), poultry (n=151) and

environment (n=160) and depicted non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al.*, 2012). The environmental samples included effluents from domestic animals, abattoirs, commercial factory effluents, crop soil, river water, and sediments. For the river water, samples were collected from a distance of at least 0.5 m from the shore and at a depth of 20 to 50 cm. The community and industrial effluents were collected at up to five different points per site using a sterile trowel and placed in a 50mL sterile falcon tube (BD, Nairobi, Kenya).

The faecal materials were obtained from the cloaca and rectum of the poultry and domestic pigs and were collected aseptically using sterile cotton swabs (Himedia, Mumbai, India). Detailed descriptions of the sampling site, sampling protocol, and sample processing have previously been described (Kimera, *et al.*, 2021 a,b). The *Escherichia coli* and *Klebsiella pneumoniae* isolates were inoculated on nutrient agar and incubated aerobically at 37°C for 24 hours prior to further processing.

DNA extraction: Briefly, a half loop of a freshly bacterial culture was suspended in 100µL of sterile DNase free water for DNA extraction. DNA was extracted by boiling in a water bath at 100°C for 10 minutes, followed by centrifugation at 1500 rpm for 3 minutes as described previously (Valat *et al.*, 2016). The supernatant containing DNA was transferred into a sterile Eppendorf PCR tube (Eppendorf AG, Hamburg, Germany) and centrifugation and separation of supernatant were repeated three times. The concentration of DNA was determined by a Nanodrop spectrophotometer (Biochrom LTD, Cambridge, England) at 260/280 wavelength (ranging from 1.5 to 1.8). The DNA was stored at -20°C, before being used for detection of resistant genes.

Screened genes: ESBL genes (*bla_{CTX-M}*, *bla_{TEM}*, and *bla_{SHV}*) and PMQR genes (*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, and *aac(6)-Ib-cr*) were the resistant genes screened. The selection of resistant genes to be screened was based on the isolates that demonstrated to be positive for ESBL and PMQR from the previous studies published in the links <https://doi.org/10.3390/antibiotics10040406> and <https://doi.org/10.3390/antibiotics10040343>. For quality check, internal quality control was performed by running each DNA sample in duplicates and for external quality control, known control organisms harboring *bla_{CTX-M}*, *bla_{TEM}*, and *bla_{SHV}* and quinolone-resistant genes (*qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *qepA*, and *aac(6)-Ib-cr*) were included in each run from DNA extraction to detection of ESBL and quinolone

resistant genes. The negative control was a DNA-free template (nuclease-free water).

PCR reaction mixture for the detection of ESBL and PMQR genes: The One Tag Master Mix Hot Start DNA polymerase kit (New England Biolabs, Ipswich, MA, USA) was used in the detection of resistance genes. Total PCR reaction volume was 25µL, consisting of One Tag Master Mix 2X Standard buffer 12.5µL, 10µM forward primer 0.5µL, 10µM reverse primer 0.5µL, nuclease-free water 9.5µL. The mixture was then aliquoted to the PCR tubes and thereafter 2µL of each DNA extract was added to the PCR tubes containing the Master Mix. The primers used in amplification of the respective resistance genes are listed in Table 1 below. The working buffer was prepared by measuring 980mL of the distilled water and mixed with 20mL of Tris-acetate –EDTA (TAE) buffer stock solution in a conical flask. Thereafter, 1.5% of the Agarose gel was prepared by measuring 1.5 gram of agarose (Merck, SA), which was then dissolved in 100mL of the working TAE buffer solution and mixed by heating in a microwave until completely melted.

To facilitate visualization of DNA 5µL gel red stain (Sigma-Aldrich, USA) was added to the agarose gel. After cooling the solution to about 60°C it was then poured into the casting tray containing sample comb and allowed to solidify at room temperature. The amplicons of each sample was mixed with 2µL loading dye and a 100-base pair marker (Thermo Scientific, (EU) Lithuania) was included for DNA band size estimation. All gels were run in 0.5xTBE buffer at 100V for 45 minutes, and visualized by UV trans-illumination. Those that produced bands were termed positive, whereas those that did not show bands were termed negative.

Statistical analysis: The data generated in this study was entered into an Excel spread sheet version Office 2010 and then transferred to Statistical package for social sciences (SPSS) version 20.0 for Windows (IBM Corp, Armonk, NY, USA) software for statistical analysis. Descriptive analysis was conducted to compare the frequencies of ESBL and PMQR genes between poultry, domestic pigs, and the environmental isolates. The Chi-square test was used to determine the difference in occurrence of resistant genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates and a p-value of less than 0.05 was considered significant.

Ethics approval: The ethical clearance for the study was issued by the National Institute for Medical Research (NIMR) of Tanzania (Reference No.

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NIMR/HQ/R.8a/Vol. IX/3133), and the Muhimbili University of Health and Allied Sciences permit no. DA.282/298/01.C. The permission to work in the

study area was sought from the Municipal Directors Office (Dar es Salaam).

Table 1. Primers and PCR conditions used for screening ESBL and PMQR resistant genes.

Gene	Primer Set and Sequence (5'-3')	Amplicon size	PCR conditions	Function	Reference
<i>bla_{CTX-M}</i>	F:SCSATGTGCAGYACAGTAA R:ACCAGAAAYVAGCGGBGC	585bp	5 min at 96°C, followed by 35 cycles of 96°C for 30s, 56°C for 40s, and at 72°C for 60s, and final incubation at 72°C for 10 min.		(Reich and Atanassova, 2013)
<i>bla_{TEM}</i>	F:ATGAGTATTCAACATTTCCG R:CTGACAGTTACCAATGCTTA	868bp 867bp	5 min at 96°C, followed by 35 cycles of 96°C for 60s, 58°C for 60s, and at 72°C for 1 min, and final incubation at 72°C for 10 min.	Hydrolytic activity of beta-lactam ring	(Lim <i>et al.</i> , 2009)
<i>bla_{SHV}</i>	F:GGTTATGCGTTATATTCGCC R:TTAGCGTTGCCAGTGCTC	867bp	5 min at 96°C, followed by 35 cycles of 96°C for 60s, 60°C for 60s, and at 72°C for 1 min, and final incubation at 72°C for 10 min.		(Lim <i>et al.</i> , 2009)
<i>qnrA</i>	F:TCAGCAAGAGGATTTCTCA R:GGCAGCACTATTACTCCCA	627bp	5 min at 94°C, followed by 32cycles of 94°C for 45s, 53°C for 1min, and at 72°C for 1min, and final incubation at 72°C for 10 min.		(Seo and Lee, 2019)
<i>qnrB</i>	F:GGMATHGAAATTCGCCACTG R:TTTGCYGYCCGCGAGTCGAA	264bp	5 min at 94°C, followed by 32cycles of 94°C for 45s, 53°C for 1min, and at 72°C for 1min, and final incubation at 72°C for 10 min.		(Castro-Sánchez <i>et al.</i> , 2016)
<i>qnrC</i>	F:GGGTTGTACATTTATTGAATC R:TCCACTTTACGAGGTTCT	447bp	5 min at 96°C, followed by 30cycles of 96°C for 30s, 56°C for 30s, and at 72°C for 1min, and final incubation at 72°C for 5 min.	Protection of target enzymes against quinolone	(Seo and Lee, 2019)
<i>rD</i>	F:CGAGATCAATTTACGGGGAATA R:AACAAGCTGAAGCGCCTG	582bp	5 min at 96°C, followed by 30cycles of 96°C for 30s, 56°C for 30s, and at 72°C for 60s, and final incubation at 72°C for 5 min.		(Seo and Lee, 2019)
<i>qnrS</i>	F:ATGGAAACCTACAATCATAAC R:AAAAACACCTCGACTTAAAGT	467bp	5 min at 94°C, followed by 32cycles of 94°C for 45s, 53°C for 1 min, and at 72°C for 1 min, and final incubation at 72°C for 10 min.		(Seo and Lee, 2019)
<i>qepA</i>	F:TGGTCTACGCCATGGACCTCA R:TGAATTCGGACACCGTCTCCG	1137bp	5 min at 96°C, followed by 30cycles of 96°C for 30s, 56°C for 30s, and at 72°C for 60s, and final incubation at 72°C for 5 min.	Decrease of quinolone concentration through efflux pump	(Zurfluh <i>et al.</i> , 2015)
<i>aac(6)-Ib-cr</i>	F:TGCGATGCTCTATGAGTGGCTA R:CTCGAATGCCTGGCGTGT	482bp	5 min at 94°C, followed by 34cycles of 94°C for 45s, 55°C for 45s, and at 72°C for 45s, and final incubation at 72°C for 10 min.	Acetylation of free nitrogen on the C7 ring	(Zurfluh <i>et al.</i> , 2015)

RESULTS AND DISCUSSION

Distribution of ESBL and quinolone resistance genes among Escherichia coli and Klebsiella pneumoniae isolates: Table 2 and Figure 1 show isolation frequencies of different ESBL and quinolone resistance genes among *Escherichia coli* and *Klebsiella pneumoniae* isolates. As shown in Fig 1, *Klebsiella pneumoniae* isolates had a lower frequency

of occurrence for *bla_{CTX-M}* but higher level of *bla_{TEM}*, compared to *Escherichia coli*, which had higher level of *bla_{CTX-M}* but less *bla_{TEM}* genes. Additionally, *Klebsiella pneumoniae* isolates had higher occurrence of quinolone resistant genes *qnrB*, *qnrS* and *aac(6)-Ib-cr*, compared to *qnrB*, *qnrS* and *aac(6)-Ib-cr* among *Escherichia coli* isolates. However, the only statistically significant difference was the higher

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occurrence of *bla*_{TEM} among *Klebsiella pneumoniae* isolates (Table 1). Figure 2 and 3 shows the gel electrophoresis bands of some of the isolates that were

positive for quinolone-resistant genes (*qnrS*) and the ESBL genes (*bla*_{TEM}).

Table 2. Frequency of ESBL and quinolone resistant genes from *Escherichia coli* and *Klebsiella pneumoniae* isolates

Resistant genes	<i>Escherichia coli</i> (n = 95)	<i>Klebsiella pneumoniae</i> (n = 35)	p-value
ESBL genes			
<i>bla</i> _{CTX-M}	26(27.4%)	7(20%)	0.392
<i>bla</i> _{TEM}	6(6.3%)	7(20%)	0.021
Quinolone resistant genes			
<i>qnrB</i>	7(7.4%)	4(11.4%)	0.461
<i>qnrS</i>	22(23.2%)	14(40%)	0.057
<i>aac(6)lb-cr</i>	5(5.3%)	2(5.7%)	0.919
<i>qepA</i>	1(1.1%)	0(0%)	0.542

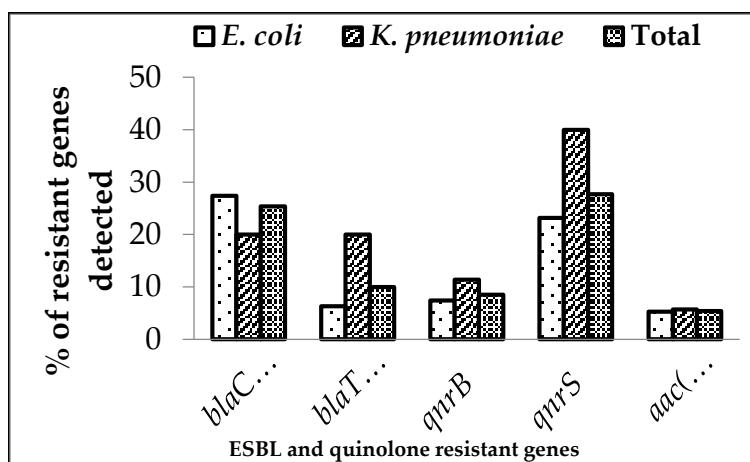


Fig 1. Distribution of ESBL and quinolone resistance genes in *Escherichia coli* (n=95) and *Klebsiella pneumoniae* (n=35) isolated from domestic pigs, poultry and environmental samples.

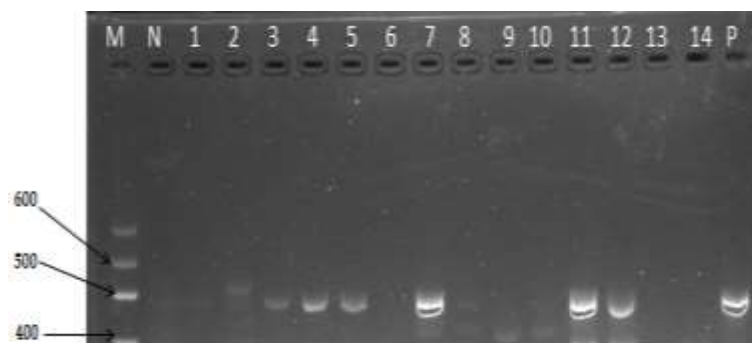


Figure 2. Gel electrophoretic bands of quinolone resistance genes (*qnrS*, 467bp). Letters M–DNA ladder, N–negative control and P–positive control. Numbers 3, 4, 5, 7, 11 and 12 are positive samples.



Figure 3. Gel electrophoretic bands of ESBL genotype (*bla*_{TEM}, 868bp). Letters M–DNA ladder, N–negative control and P–positive control. Numbers 7, 12 and 14 are positive samples.

Distribution of ESBL and quinolone resistance genes in isolates from domestic pigs, poultry and environmental samples: As shown in Table 3, occurrence of resistance genes were highest in isolates from the environmental samples (86%, 33/38), followed by poultry (72.5%, 29/40), and domestic pigs (21.4%, 9/52). The most frequent resistance gene was

qnrS, followed by *bla_{CTX-M}* and *bla_{TEM}*. The occurrence of *bla_{TEM}* was significantly higher in environmental (18.4%, 7/38) and poultry (15.8%, 6/40) but not found in domestic pigs. Significant differences were also observed for *qnrS*, in poultry, environment and domestic pigs, respectively.

Table 3. Distribution of ESBL and quinolone resistance genes isolated from domestic pigs, poultry, and environmental samples

Resistant genes	Sample source			p-value
	Domestic pigs (n=52)	Poultry (n=40)	Environment (n=38)	
<i>bla_{CTX-M}</i>	11(20.4%)	15(39.5%)	7(18.4%)	0.059
<i>bla_{TEM}</i>	0(0%)	6(15.8%)	7(18.4%)	0.005
<i>qnrB</i>	4(7.4%)	2(5.3%)	5(13.2%)	0.436
<i>qnrS</i>	6(11.1%)	18(47.4%)	12(31.6%)	0.001
<i>aac(6)lb-cr</i>	1(1.9%)	4(10.5%)	2(5.3%)	0.192
Combinations of 2, 3 and 4 genes	3(5.7%)	12(30%)	6(15.8%)	0.013

Co-occurrence of multiple resistant genes: About 17.7% (23/130) of the *Escherichia coli* and *Klebsiella pneumoniae* isolates from poultry, domestic pigs and the environment samples depicted co-existence of ESBL and quinolone resistance genes (Table 4).

Combination of *bla_{CTX-M}* + *qnrS* in *Klebsiella pneumoniae* and *bla_{TEM}* + *qnrS* both in *Escherichia coli* and *Klebsiella pneumoniae* had highest occurrence.

Table 4. Co-existence of ESBL and quinolone resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolates.

Resistant genes	<i>Escherichia coli</i> (n = 95)	<i>Klebsiella pneumoniae</i> (n = 35)	Total
<i>bla_{TEM}</i> + <i>qnrS</i>	2(2.1%)	2(5.7%)	4
<i>bla_{CTX-M}</i> + <i>qnrS</i>	2(2.1%)	4(11.4%)	6
<i>bla_{CTX-M}</i> + <i>qnrB</i>	3(3.2%)	0(0%)	3
<i>qnrB</i> + <i>qnrS</i>	1(1.1%)	0(0%)	1
<i>aac(6)lb-cr</i> + <i>qnrS</i>	0(0%)	1(2.9%)	1
<i>bla_{CTX-M}</i> + <i>aac(6)lb-cr</i> + <i>qnrS</i>	4(4.2%)	0(0%)	4
<i>bla_{TEM}</i> + <i>qnrB</i> + <i>qnrS</i>	0(0%)	1(2.9%)	1
<i>bla_{CTX-M}</i> + <i>qnrB</i> + <i>qnrS</i>	1(1.3%)	0(0%)	1
<i>bla_{CTX-M}</i> + <i>bla_{TEM}</i> + <i>qnrS</i>	1(1.1%)	0(0%)	1
<i>bla_{CTX-M}</i> + <i>bla_{TEM}</i> + <i>qnrB</i> + <i>qnrS</i>	0(0%)	1(2.9%)	1

This study aimed to determine the occurrence of ESBL and PMQR genes among *Escherichia coli* and *Klebsiella pneumoniae* isolates obtained from poultry, domestic pigs and the environment in the Msimbazi river basin the most densely populated area in Tanzania (Kayombo and Mayo, 2018; Sawe *et al.*, 2019). The area has very high anthropogenic activities including high concentration of pharmaceutical and commercial industries, hospital, veterinary and human drug sellers, crops and animal farming with intense use of pesticides and antimicrobials and environmental contamination that have been linked with antimicrobial use and resistance (Kayombo and Mayo, 2018; Sawe *et al.*, 2019). We hypothesized that these anthropogenic activities may lead to the occurrence and spread of antimicrobial resistance genes including those encoding for ESBL production and PMQR as previously reported (Alonso *et al.*, 2017; Salah *et al.*, 2019). The most commonly detected ESBL genes were

bla_{CTX-M}, while quinolone resistance genes were *qnrS*, *qnrB* and *aac(6)-lb-cr*. This finding is in line with those of other studies (Athanasakopoulou *et al.*, 2021; Kammili *et al.*, 2020; Moremi *et al.*, 2021; Seni *et al.*, 2017) which reported predominance of *bla_{CTX-M}* and *qnrS* in isolates from human, animals and environment in *Escherichia coli* and *Klebsiella pneumoniae* species. This finding suggests serious concern given the fact that these beta-lactams and quinolone classes of antimicrobials are the affordable and most commonly used in treatment, prophylaxis and metaphylaxis, with limited alternative options (Mdegela *et al.*, 2021; Rasmussen *et al.*, 2015) The observed level and pattern of resistance imply that most of currently used antimicrobials in animal infections might be ineffective for their intended use and may contribute to the emergence and spread of resistant bacteria causing infections that are difficult to treat both in human and animal. We did not find the

ESBL genes *bla*_{SHV} and quinolone resistant genes *qnrA*, *qnrC* and *qnrB*, which have been reported by other studies (Alouache *et al.*, 2014; Fortini *et al.*, 2011; Salah *et al.*, 2019). These variations between and within countries could be due to differences in the extent and types of antimicrobial usage in animal production, variations in policies and regulations regarding usage and disposal of antimicrobial agents as well as differences in public awareness, warrant geographic specific surveillance of AMR (Ejaz *et al.*, 2021; Ohene Larbi *et al.*, 2021). We found out that *Escherichia coli* had a slightly higher level of ESBL genes *bla*_{CTX-M} compared to *Klebsiella pneumoniae* isolates, the variations that are supported by the previous studies (Athanasakopoulou *et al.*, 2021; Salah *et al.*, 2019). Higher level of *bla*_{CTX-M} in *Escherichia coli* than *Klebsiella pneumoniae* isolates found in the present study is consistent with that done in Ghana (Georgina Solano-Gálvez *et al.*, 2021). On the contrary, *Klebsiella pneumoniae* isolates had higher occurrence of quinolone resistance genes *qnrB*, *qnrS* and *aac* (6)-*lb-cr*, compared to *Escherichia coli* isolates, a finding which is consistent with that of isolates from chicken and domestic pigs in Nigeria (Fortini *et al.*, 2011). These variations could be associated with intrinsic differences in antimicrobial resistant genes (ARGs) expression (Wyres and Holt, 2018). We observed co-existence of ESBL and quinolone resistance genes in some of the isolates from poultry, domestic pigs and the environmental samples, with the most frequent combination of *bla*_{CTX-M} + *qnrS* in *Klebsiella pneumoniae* and *bla*_{TEM} + *qnrS* both in *Escherichia coli* and *Klebsiella pneumoniae*, and *bla*_{CTX-M} + *aac*(6)*lb-cr* + *qnrS* in *Escherichia coli*, as has been reported by others studies (Alouache *et al.*, 2014; Ben Sallem *et al.*, 2012). Similar observations have been reported in Togo and Algeria where co-existence of ESBL and quinolone resistant genes among *Escherichia coli* and *Klebsiella pneumoniae* isolates collected from human specimens, cattle, and environment (Alouache *et al.*, 2014; Salah *et al.*, 2019) which has been attributed to shared resistance mechanisms (Alouache *et al.*, 2014; Samreen *et al.*, 2021). The co-existence of resistant genes plays a potential role in the transmission of resistant genes that calls for across human and animal compartments thus highlighting the importance of collaborative One Health efforts to address the threat. Regarding sample sources, isolates from environmental samples had higher level of resistant genes, followed by poultry and domestic pigs indicating the role of environment as being a reservoir of ARGs originating from human anthropogenic activities (Ben Said *et al.*, 2016; Fletcher, 2015; Ram and Kumar, 2020).

This study which has provided useful insights on the occurrence of ESBL and quinolone resistance genes in poultry, domestic pigs and environment has some limitations. The use of conventional PCR, instead of whole genome sequencing (WGS) limited information on genetic relatedness and unveiling of potential novel resistance mechanisms of the isolates. Secondly, screening of all resistant genes encoding for ESBL production and PMQR such as *bla*_{AmpC}, VEB, OXA, PER, *oqxA*, and *oqxB* was not done and this might have underestimated level of ESBL and PMQR genes across poultry, domestic pigs and the environment compartments. Nonetheless, results of this investigation provides a foundation for more detailed molecular studies, given the paucity of such studies, especially those involving environmental surveillance in the region.

Conclusions: The wide-spread presence of several genes encoding for quinolone resistance and ESBL production isolates recovered from this study certainly contributes to the growing burden of AMR in the study area, with adverse clinical outcomes, economic and societal impact. The interactions between animals, humans and the environment are likely to be responsible for occurrence of resistant bacteria. We therefore recommend interventions that should focus on the implementation of comprehensive and well-coordinated One Health approach.

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