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Occurrence of EBSL and quinolone resistance genes among Escherichia coli and Klebsiella pneumoniae isolated from poultry, domestic pigs and environment in the Msimbazi River Basin in Tanzania

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Abstract

Background: The Enterobacteriaceae family particularly *Escherichia coli* and *Klebsiella pneumoniae* that are resistant to both quinolones and beta lactams occur worldwide, causing wide spread infections that are difficult to treat. We investigated 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.

Methods: A total of 110 non- duplicated isolates obtained from the poultry (n = 30), domestic pigs (n = 42) and environment (n = 39) were screened for ESBLs genes (CTX-M, TEM, and SHV) and plasmid mediated quinolone resistance (PMQR) genes (*qnrA, qnrB, qnrS, qnrC, qnrD, qepA*, and *aac(6)-Ib-cr*) using polymerise chain reaction (PCR).

Results: The most commonly detected ESBL genes were *blaCTX-M* (31.5%, n=41) and *blaTEM* (10%, n=13), while quinolone resistance genes were *qnrS* (27.7%, n=36), *qnrB* (6.9%, n=9) and *aac (6)-Ib-cr* (5.4%, n=7). ESBL gene *blaSHV* 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 *blaCTX-M* and *blaTEM*. About 17.7% (n=23) of the isolates depicted co-existence of ESBL and quinolone resistance genes.

Conclusion: 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.

1. Introduction

In Tanzania, the demand for short cycled food animal such as poultry and domestic pigs has increased in recent years [1, 2]. 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 [3]. This high demand has led to intensive production methods, associated with poor animal husbandry practices, overcrowding, high disease frequency and overstretched veterinary extension services [2, 4–7].

The antimicrobial usage in animal sector in Tanzania from 2010 to 2017 amounted to 12,147,491 kilograms [8]. The most commonly used antimicrobial agents were tetracycline, sulphonamides and trimethoprim, quinolones, aminoglycosides, and beta-lactams [2, 4, 5, 8, 9]. Tetracycline alone accounted for 66.6% (8,090,798 kg) of the total drugs [8]. 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 [10, 11] and the sorrounding environment [7, 12].

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 development and spread of antimicrobial resistance genes, which can be transferred across animal, human and environment compartments as shown in a previous study [12–16]. Indeed, conjugative plasmids have been responsible for disseminating CTX-M-15 among human, animals and the environment [13]. Additionally, the resistant genes *blaCTX-M*, *qnrS* and *aac(6)-Ib-cr* were found to circulate between human, domestic and companion animals, fish and environment [12, 14, 15, 17]. In Uganda similar genotypes of *Salmonella enterica serovar* Kentucky, were found in poultry, human and environmental sources that suggested zoonotic and environmental transmission [17]. In Tunisia a study by [18] identified IncH12-type plasmid spreading the *blaCTX-M-1* and mcr-1 in farm chick, which were reported in cattle in France and food sample in Portugal [19]. In Nigeria detected the Plasmid Mediated Quinolone Resistance (PMQR) from *Escherichia coli* strains recovered from health pigs and poultry that resembled those from human origin [20]. Furthermore, spread of similar antimicrobial resistance genes from community, human, animals and environment have been reported in Tanzania, Cameroon, Nigeria, Angola and Netherlands [21–26]. A recent review reported that humans exposed to poultry farms carried Carbapenem-Resistant Enterobacteriaceae (CRE) with close relation from the environmental isolates [27].

Members of the Enterobacteriaceae family particularly *Escherichia coli* and *Klebsiella pneumoniae* that are resistant to both quinolones and beta lactams occur worldwide [21, 22], causing wide spread infections that are difficult to treat [28]. Molecular characterization of antimicrobial resistance genes offer a deeper insight to the possible mechanisms of transmission, an important source of information in the control of AMR spread [29, 30]. Some of the most commonly involved resistance genes include TEM, SHV, and 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 [26, 31–36]. These genes are placed on the plasmids and mobile genetic elements, which make their transmission extremely easy [13, 29, 37, 38].

The Msimbazi river basin in Tanzania has a myriad of human activities making it a hot spot for the spread AMR bacteria and resistant genes between human-animal-environmental compartments [39–42]. 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 [43, 44]. However, all these studies were limited to phenotypic screening of antibiotic resistant pathogens and none focused on the occurrence of resistant genes. The aim of this study was to determine the occurrence of ESBL and quinolone resistant genes from *E. coli* and *K. pneumoniae* isolates recovered from poultry, domestic pigs and environment in Tanzania and to further characterize them using molecular methods for comparison.

2. Materials And Methods

2.1. Bacteria isolates

A total of 130 non-duplicate multidrug resistant *E. coli* (95) and *K. pneumonia*e (35) isolates from poultry (40), domestic pigs (52) and environment (38) was selected from stored isolates obtained between July 2020 and April 2021 [43, 44]. These isolates were randomly selected from a total of 504 isolates from domestic pigs (193), poultry (151) and environment (160) and depicted resistance to more than three different classes of antibiotics [45]. The environmental samples included effluents from domestic animals, abattoir, and 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 50 mL sterile falcon tube (BD, Nairobi, Kenya). The faecal materials were obtained from 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 [43, 44]. The *E. coli* and *K. pneumoniae* isolates were inoculated on nutrient agar and incubated aerobically at 37 ^{o}C for 24 hours prior to further processing.

2.2. DNA extraction

Briefly, a half loop of a freshly bacterial culture was suspended in 100 µL of sterile DNAse free water for DNA extraction. DNA was extracted by boiling in a water bath at 100 ^{o}C for 10 minutes, followed by centrifugation at 1500 rpm for 3 minutes as described previously [46]. 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 ^{o}C, before being used for detection of resistant genes.

2.3. Screened genes

ESBL genes (CTX-M, TEM, and SHV) and PMQR genes (*qnrA, qnrB, qnrS, qnrC, qnrD, qepA*, and *aac(6)-Ib-cr* were the resistant genes screened. 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.The positive controls used were the *E. coli* and *K. pneumoniae* strains available at the university research laboratory and the negative control was a DNA free template (nuclease-free water).

2.4. PCR reaction mixture for the detection of ESBL and plasmid mediated quinolone resistant (PMQR) genes

The One Tag Master Mix Hot Start DNA polymerase kit (New England Biolabs, Ipswich, MA, USA) was used in 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 980 mL of

the distilled water and mixed with 20 mL 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 100 mL of the working TAE buffer solution and mixed by heating in a microwave until completely melted. To facilitate visualization of DNA 5 µL gel red stain (Sigma-Aldrich, USA) was added to the agarose gel. After cooling the solution to about 60 ^{O}C it was then poured to 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 as negative.

2.5 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 *E. coli* and *K. pneumoniae* isolates and a p-value of less than 0.05 was considered significant.

2.6 Ethical considerations

Ethical clearance for this study was issued i) the Tanzania Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR) (Reference No. NIMR/HQ/R.8a/Vol. IX/3133), and ii) the Muhimbili University of Health and Allied Sciences (MUHAS) Institutional Review Board (Permit No. DA.282/298/01.C).

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
blaCTX-M	F:SCSATGTGCAGYACCAGTAA R:ACCAGAAYVAGCGGBGC	585 bp	5 min at 96°C, followed by 35 cycles of 96°C for 30 s, 56°C for 40 s, and at 72°C for 60 s, and final incubation at 72°C for 10 min.	Hydrolytic activity of beta lactam ring	[33, 47, 48]
blaTEM	F:ATGAGTATTCAACATTTCCG R:CTGACAGTTACCAATGCTTA	868 bp	5 min at 95°C, followed by 35 cycles of 94°C for 30 s, 56°C for 40 s, and at 72°C for 1min s, and final incubation at 72°C for 10 min.		[49]
blaSHV	F:GGTTATGCGTTATATTCGCC R:TTAGCGTTGCCAGTGCTC	867 bp	5 min at 95°C, followed by 35 cycles of 94°C for 30 s, 56°C for 40 s, and at 72°C for 1min s, and final incubation at 72°C for 10 min.		[49]

Gene	Primer Set and Sequence (5'-3')	Amplicon size	PCR conditions	Function	Reference
qnrA	F:TCAGCAAGAGGATTTCTCA R:GGCAGCACTATTACTCCCA	627 bp	5 min at 94°C, followed by 32cycles of 94°C for 45s, 53°C for 1min, and at 72°C for 1min s, and final incubation at 72°C for 10 min.	Protection of target enzymes angainst quinolone	[28, 50, 51]
qnrB	F:GGMATHGAAATTCGCCACTG R:TTTGCYGYYCGCCAGTCGAA	264 bp	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.		[50]
qnrC	F:GGGTTGTACATTTATTGAATC R:TCCACTTTACGAGGTTCT	447 bp	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.		[52]

Gene	Primer Set and Sequence (5'-3')	Amplicon size	PCR conditions	Function	Reference
qnrD	F:CGAGATCAATTTACGGGGAATA R:AACAAGCTGAAGCGCCTG	582 bp	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.		[52]
qnrS	F:ATGGAAACCTACAATCATAC R:AAAAACACCTCGACTTAAGT	467 bp	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.		[50]
qepA	F:TGGTCTACGCCATGGACCTCA R:TGAATTCGGACACCGTCTCCG	1137 bp	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	[51, 52]

Gene	Primer Set and Sequence (5'-3')	Amplicon size	PCR conditions	Function	Reference
aac(6)-Ib-cr	F:TGCGATGCTCTATGAGTGGCTA R:CTCGAATGCCTGGCGTGTTT	482 bp	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	[51, 52]

3. Results

3.1. Distribution of ESBL and quinolone resistance genes among E. coli and K. pneumoniae isolates

Table 2 and Fig. 1 show isolation frequencies of different ESBL and quinolone resistance genes among *E. coli* and *K. pneumoniae* isolates. As shown in Fig. 1, K. *pneumoniae* isolates had a lower frequency of occurrence for *blaCTX-M* (20%%) but higher level of *blaTEM* (20%), compared to *E. coli*, which had higher level of *blaCTX-M* (27.4%) but less *blaTEM* genes (6.3%). Additionally, *K. 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 *E. coli* isolates. However, the only statistically significant difference was the higher occurrence of *blaTEM* among *K. pneumoniae* isolates (Table 1). Figures 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 (*blaTEM*).

Table 2
Frequency of ESBL and quinolone resistant genes from *E. coli* and *K. pneumoniae* isolates.

Resistant genes	*E. coli* (n = 95)	*K. pneumoniae* (n = 35)	P-value
ESBL genes			
blaCTX-M	26(27.4%)	7(20%)	0.392
blaTEM	6(6.3%)	7(20%)	0.021
Quinolone resistant genes			
qnrB	7(7.4%)	4(11.4%)	0.461
qnrS	22(23.2%)	14(40%)	0.057
aac(6)Ib-cr	5(5.3%)	2(5.7%)	0.919
qepA	1(1.1%)	0(0%)	0.542

3.2. 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 was highest in isolates from the environmental samples (86%, n = 33), followed by poultry 72.5% (n = 29), and domestic pigs 21.4%, (n = 9). The most frequent resistance gene was *qnrS*, followed by *blaCTX-M* and *blaTEM*. The occurrence of *blaTEM* was significantly higher in environmental (18.4%) and poultry (15.8%) but not found in domestic pigs. Significant differences were also observed for *qnrS*, being 47.4%, 31.6% and 11.1% in poultry, environment and domestic pigs, respectively. On the other hand, differences in *blaCTX-M, qnrB*, and *aac(6)Ib-cr* were not statistically different.

Table 3
Distribution of ESBL and quinolone-resistance genes in *E. coli* and *K. pneumoniae* isolated from domestic pigs, poultry and environmental samples

Resistant genes	Sample source			
	Domestic pigs (n = 52)	Poultry (n = 40)	Environment (n = 38)	P = value
blaCTX-M	11(20.4%)	15(39.5%)	7(18.4%)	0.059
blaTEM	0(0%)	6(15.8%)	7(18.4%)	0.005
qnrB	4(7.4%)	2(5.3%)	5(13.2%)	0.436
qnrS	6(11.1%)	18(47.4%)	12(31.6%)	0.001
aac(6)Ib-cr	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

3.3. Co-occurrence of multiple resistant genes

About 17.7% (n = 23) of the *E. coli* and *K. pneumoniae* isolates from poultry, domestic pigs and the environment samples depicted co-existence of ESBL and quinolone resistance genes (Table 4). Combination of *blaCTX-M* + *qnrS* in *K. pneumoniae* and *blaTEM* + *qnrS* both in *E. coli* and *K. pneumoniae* had highest occurrence.

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

Resistant genes	*E. coli* (n = 95)	*K. pneumoniae* (n = 35)	Total
blaTEM + *qnrS*	2(2.1%)	2(5.7%)	4
blaCTX-M + *qnrS*	2(2.1%)	4(11.4%)	6
blaCTX-M + *qnrB*	3(3.2%)	0(0%)	3
qnrB + *qnrS*	1(1.1%)	0(0%)	1
aac(6)lb-cr + *qnrS*	0(0%)	1(2.9%)	1
blaCTX-M + *aac(6)lb-cr* + *qnrS*	4(4.2%)	0(0%)	4
blaTEM + *qnrB* + *qnrS*	0(0%)	1(2.9%)	1
blaCTX-M + *qnrB* + *qnrS*	1(1.3%)	0(0%)	1
blaCTX-M + *blaTEM* + *qnrS*	1(1.1%)	0(0%)	1
blaCTX-M + *blaTEM* + *qnrB* + *qnrS*	0(0%)	1(2.9%)	1

4. Discussion

This study aimed to determine the occurrence of ESBL and PMQR genes among *E. coli* and *K. pneumoniae* isolates obtained from poultry, domestic pigs and the environment in the Msimbazi river basin the most densely populated area in Tanzania [40, 53]. 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 AMU and AMR [40, 42, 53, 54]. We hypothesized that these anthropogenic activities may lead to occurrence and spread of antimicrobial resistance genes including those encoding for ESBL production and PMQR as previously reported [31, 55, 56].

The most commonly detected ESBL genes were *blaCTX-M*, while quinolone resistance genes were *qnrS, qnrB* and *aac (6)-lb-cr*. This findings is in line with those of other studies [12, 33, 57–60] which reported predominance of *blaCTX-M* and *qnrS* in isolates from human, animals and environment in *E. coli* and *K. pneumoniae* species. This finding suggests serious concern given the fact that these antibiotic classes are the affordable and most commonly used in treatment, prophylaxis and metaphylaxis, with limited

alternative options [2, 4, 61]. The observed level and pattern of resistance imply that most of currently used antibiotics 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.

We did not find the ESBL genes *blaSHV* and quinolone resistant genes *qnrA*, *qnrC* and *qnrB*, which have been reported by others [20, 29, 31]. 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 [62, 63].

We found out that *E. coli* had slightly higher level of ESBL genes *blaCTX-M* compared to *K. pneumoniae* isolates, the variations that is supported by the previous studies whereby (51.6%) of the MDR *E. coli* were ESBL producers compared to (49.5%) of the *K. pneumoniae* isolates [43, 44]. Higher level of *blaCTX-M* in *E. coli* than *K. pneumoniae* isolates found in the present study is consistent with that done in Ghana [64]. On the contrary, *K. pneumoniae* isolates had higher occurrence of quinolone resistance genes *qnrB*, *qnrS* and *aac (6)-Ib-cr*, compared to *E. coli* isolates, a finding which is consistent with that of isolates from chicken and domestic pigs in Nigeria [20]. These variations could be associated with intrinsic differences in antibiotic resistant genes (ARGs) expression [65, 66].

We observed co-existence of ESBL and quinolone resistance genes in some of the isolates, with the most frequent combination of *blaCTX-M + qnrS in K. pneumoniae* and *blaTEM + qnrS* both in *E. coli* and *K. pneumoniae* and *blaCTX-M + aac(6)Ib-cr + qnrS* in *E. coli*, as has been reported by others [29, 43, 67]. Similar observations have been reported in Togo and Algeria where co-existence of ESBL and quinolone resistant genes among *E. coli* and *K. pneumoniae* isolates collected from human specimens, cattle, and environment [29, 31] which has been attributed to shared resistance mechanisms [29, 31, 68].

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 [69–71].

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 *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.

5. Conclusion

The wide-spread presence of several genes encoding for quinolone resistance and ESBL production among *E. coli* and *K. pneumoniae* isolates recovered from pigs, poultry and environment certainly contribute to the growing burden of AMR in the Msimbazi River Basin, with adverse clinical outcomes, economic and societal impact. The complex interactions between animals, humans and the shared environment are likely to be responsible for the wide spread of occurrence of resistance bacteria. We therefore recommend interventions that should focus on implementation of comprehensive and well-coordinated One Health approach. These interventions should include continuous surveillance and stewardship of AMU and AMR in humans, animals and the environment, emphasis on implementation of regulations governing handling, distribution and sale of human and veterinary medicines, proper disposal of waste and effluent and improved agriculture and animal farming practices along the river basin. Modelling will be required to determine the most effective set of approaches to reduce the burden of AMR in the study area.

Abbreviations

AMR
Antimicrobial resistance
AMU
Antimicrobial use
ARGs
Antibiotic resistance genes
DNA
CRE:Carbapenem resistance enterobacteriaceae
CTX-M
cefotaximase Munich
DNA
Deoxyribo nucleic acid
EDTA
Ethylenediamine tetraacetic acid
ESBL
Extended spectrum beta lactamase
IMP
Imipenem metallo beta lactamase
KPC
Klebsiella pneumoniae carbapenemase
NDM
New Delhi metallo beta lactamase
OXA
Oxacillinases
PCR

Polymerise chain reaction
PMQR
Plasmid mediated quinolone resistance
SHV
Sulfhydril variable
SPSS
Statistical package for social sciences
TEM
Temoniera
VIM
Verona integrin-encoded metallo beta lactamase
WGS
Whole genome sequencing.

Declarations

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Authors' contributions:ZIK, conducted study design, sample collection, laboratory work and analysis, original draft preparation; FXM, organized and supervised laboratory work; SEM, coordinated data analysis, interpretation and manuscript editing; EDK, reviewed and edited the manuscript; MINM, designed the outline, reviewed the manuscript, and supervised writing of the manuscript. All authors have read and agreed approved the final version of the manuscript.

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Ethics approval and consent to participate: The ethical clearance was provided by the Medical Research Coordinating Committee of the National Institute for Medical Research (NIMR) of Tanzania (Reference No. NIMR/HQ/R.8a/Vol. IX/3133), and Muhimbili University of Health and Allied Sciences (Permit No. DA.282/298/01.C). The permission was sought from the relevant authorities that are the municipal directors at the three districts. There is no participation section for this study as it is not applicable.

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Figures

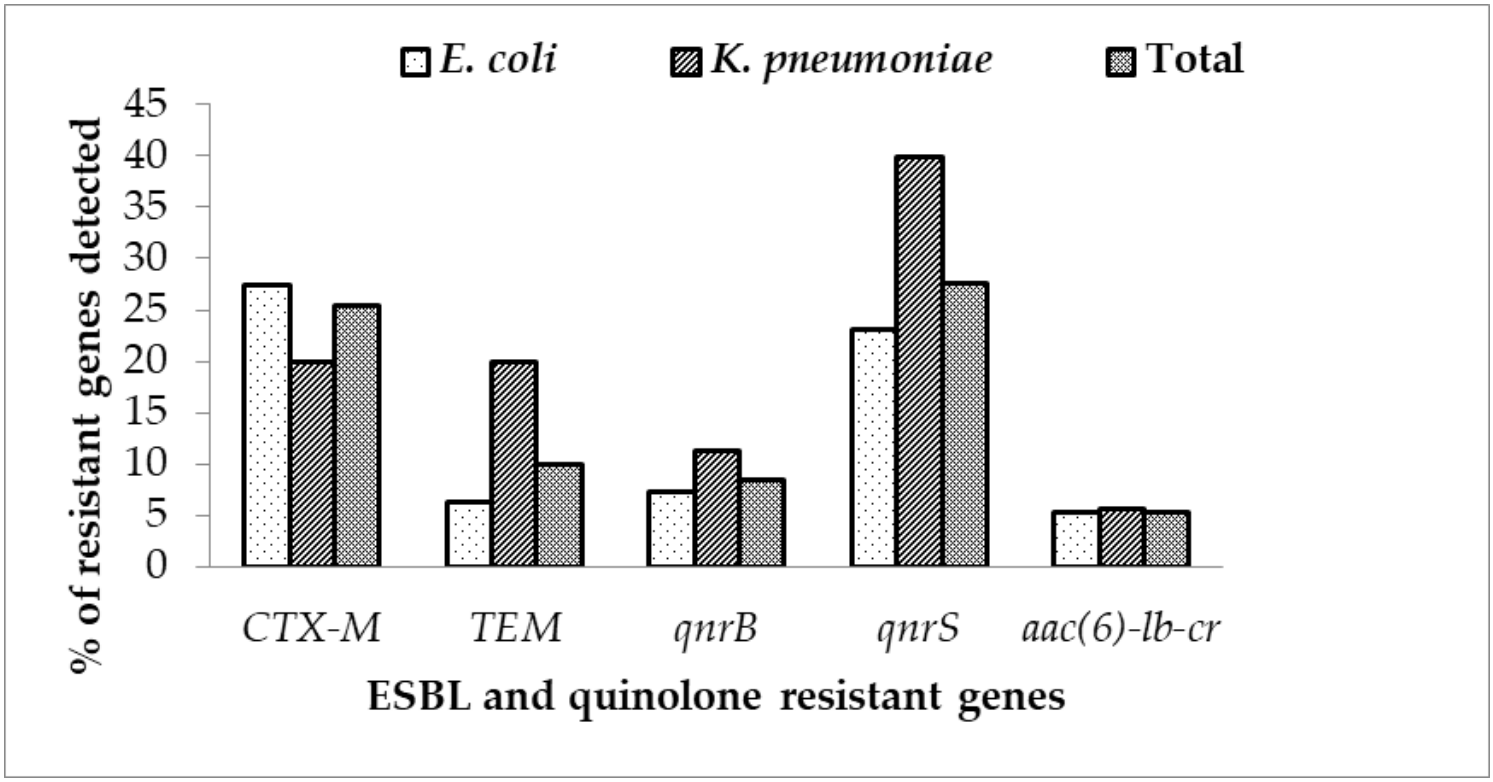


Figure 1

Distribution of ESBL and quinolone resistance genes in *E. coli* and *K. pneumoniae* isolated from domestic pigs, poultry and environmental samples.

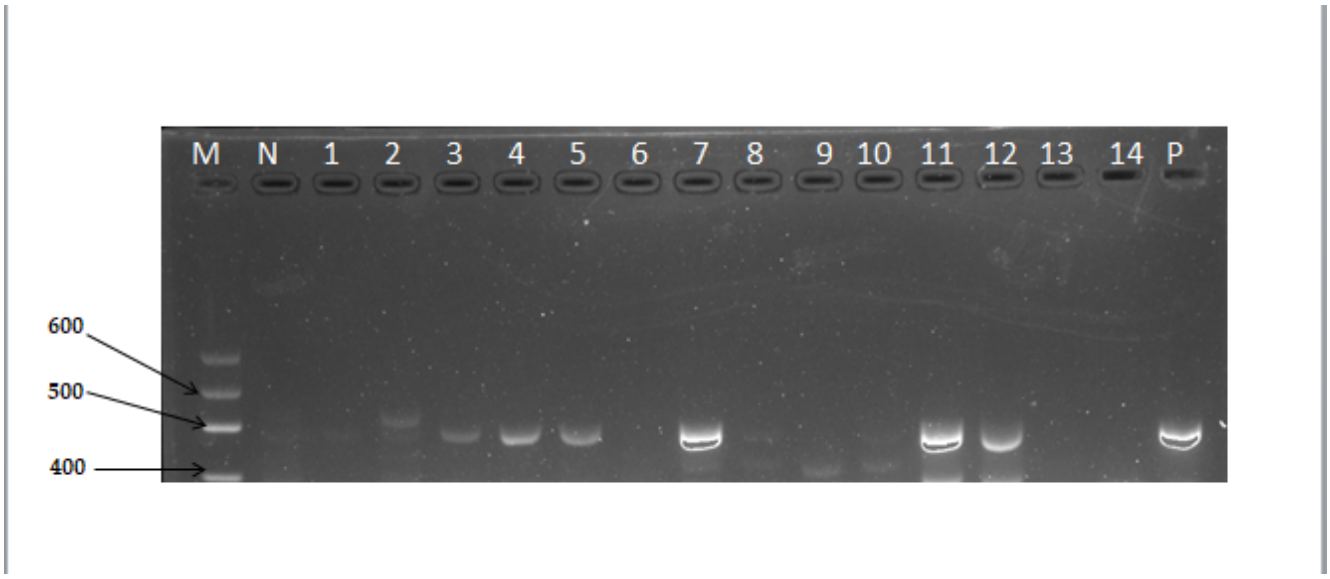


Figure 2

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

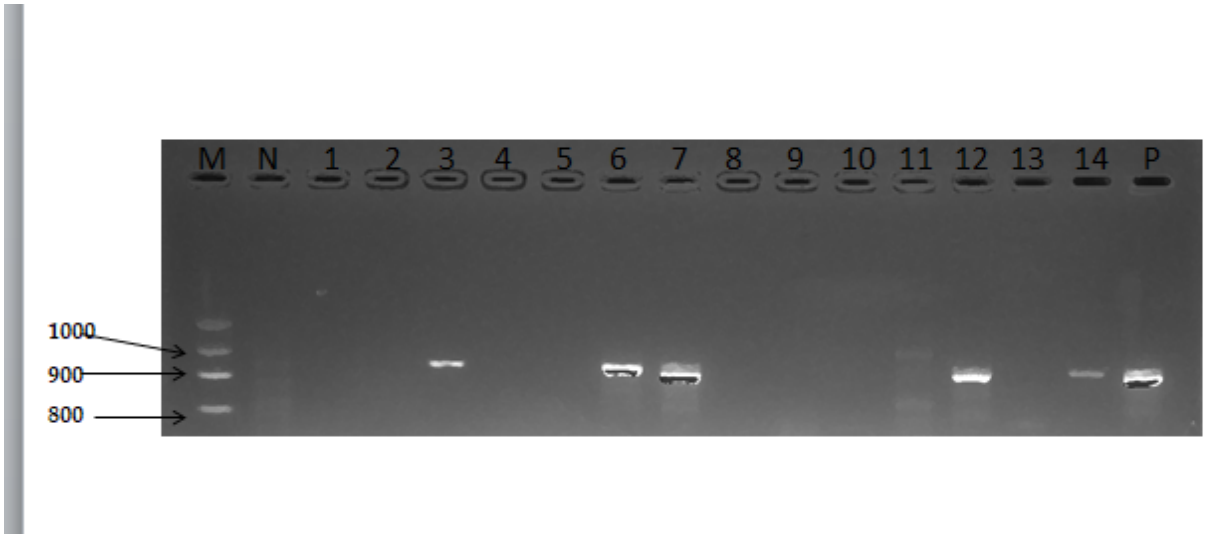


Figure 3

Gel electrophoretic bands of ESBL genotype (*blaTEM*). Letters M – DNA ladder, N – negative control and P – positive control. Numbers7, 12 and 14 are positive samples.