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Prevalence of multidrug resistant extended spectrum β-lactamase producing *E. coli* in live chicken and chicken products in Zanzibar

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Abstract

Introduction: *antimicrobial resistance due to the emergence of Extended Spectrum Beta Lactamase Escherichia coli (ESBL E. coli) is a global health threat. Chicken is a known reservoir and contributes to the dissemination of ESBL producing E. coli in the food production chain as documented by studies conducted elsewhere including Tanzania Mainland. However, information is scarce in Zanzibar. The current study aimed to investigate the prevalence of multidrug resistant ESBL producing E. coli in chicken and chicken products.* ***Methods:*** *a cross-sectional study was conducted from March to September 2022. Samples were randomly collected in Mwanakwerekwe market. The ESBL producing E. coli were identified using conventional bacterial culture and biochemical tests. The Kirby-Bauer disk diffusion method was used to assess the antimicrobial susceptibility and Multidrug Resistance (MDR) of the isolates tested against six classes of antibiotics.* ***Results:*** *overall, the prevalence of ESBL E. coli was 39(14.9%), 18(46.2%) among broilers, 15(38.5%) in layers, and 6(38.5%) in indigenous chicken (p>0.05). In total, 10(35.7%) isolates were recovered from imported frozen broiler meat and 18(64.3%) from fresh local broiler meat. A high level of antimicrobial resistance of the isolates was observed against tetracycline 38(97.6%), ampicillin 39(97.6%), and sulfamethoxazole/trimethoprim 31(79.2%). All isolates showed full susceptibility to amoxicillin-clavulanate and meropenem. A high resistance rate was observed among all ESBL producing E. coli strains isolated in broiler chicken (42.8%-100%).* ***Conclusion:*** *multidrug resistance of ESBL producing E. coli was prevalent in live chicken and chicken products in Zanzibar. High levels of antimicrobial resistance of ESBL producing E. coli were observed against commonly used antibiotics for the treatment of poultry infection. It is important to improve surveillance of ESBL producing E. coli in poultry industries.*

Introduction

Poultry farming is extremely important in terms of food security and nutrition, especially in developing countries [1]. Antimicrobial agents are commonly used for the treatment and prophylaxis of microbial diseases [2]. However, over- and irrational use of antibiotics in food animal's production are primary sources of developing resistant bacteria [3]. *E. coli* is a normal microflora found in the intestine of animals and humans and is a major cause of public health threat [4]. Worldwide, *E. coli* is associated with foodborne illness especially in developing countries. It is estimated to cause 48 million morbidity, 128000 to be hospitalized and 3000 deaths [5].

Extended Spectrum β-lactamase-producing *E. coli* (ESBL producing *E. coli*) are zoonotic in nature and among the commonest resistant pathogens in chicken [6]. The ESBL producing *E. coli* produces the β-lactamase enzymes that inactivate the beta-lactam antibiotics [7]. It is necessary to monitor the spread of ESBL *E. coli* pathogens from animals and food products to humans and environment, since the infections generated by ESBL producing *E. coli* pose a significant threat to health care setting [3,4]. The emergence of the ESBL producing *E. coli* in animal food products accelerates the magnitude of antibiotic resistance with specific concern to beta-lactam antibiotics [8]. The pathogens resistant to commonly used antibiotics pose a serious challenge in poultry farming [9]. Studies have shown that chicken and chicken products are the potential reservoirs of ESBL producing *E. coli* [10].

Moreover, multidrug resistance has increased globally as a result of increasing prevalence of ESBL producing *E. coli* associated with growing reservoirs such as chicken and irrational use of antibiotics [11]. Prevalence of ESBL producing *E. coli* in chickens has recently been reported in China [12], in Germany 74.8% [13] in Egypt 25.3% [14] and Tanzania 50.5% [15]. Chicken is a known reservoir and contributes in dissemination of zoonotic pathogens including ESBL producing *E. coli* along food production chain [16]. Antibiotics play an important role in prophylaxis and treatment of human and veterinary bacterial infections [4]. However, the irrational use of the antibiotics has generated a selective pressure that leads to globally multiple drug-resistant microbial populations [16]. Multiple Antibiotic Resistance (MAR) indexing is a method that is widely used to track the bacterial source. The MAR index values greater than 0.2, indicate a high risk of contamination by multidrug resistant bacteria, where antibiotics are often used [17]. The magnitude of the antimicrobial resistance problem is potentially higher in developing countries, where the level of infectious diseases is high and this corresponds to a higher use of broad-spectrum antibiotics [8].

In Zanzibar there are no available data that show the occurrence of ESBL *E. coli* and their antibiotics resistance in chicken and chicken products. The current study aimed to investigate the prevalence of multidrug resistant ESBL producing *E. coli* in live chicken and their products in Zanzibar. The findings of this study provide baseline evidence for the implementation of the Zanzibar Antimicrobial Resistance Action Plan and One Health Strategic Plan in the mitigation of antimicrobial resistance and optimizing rational use of antibiotics in the treatment of ESBL producing *E. coli*.

Methods

Study design: a cross-sectional study was carried out covering nine months; from March to September 2022.

Setting: the study was conducted at Mwanakwerekwe Chicken Market located in West A district of Unguja Island in Zanzibar. The West A district had a population of 245,889 at the time of data collection [18]. Mwanakwerekwe Market is the main chicken market in Zanzibar visited by many Zanzibaris, thus was an appropriate area for the study. In addition, all chicken farmers from rural and urban areas of all districts in Zanzibar use this market to sell their chicken.

Participants: the study investigated healthy local broiler and layer chickens that were up for sale and intended for meat in the market. The sick chickens and those administered with antibiotics within the past seven days were excluded from the study.

Variables: prevalence of ESBL producing *E. coli* was considered as the independent variable and the rate of multidrug resistance was a dependent variable.

Data source/measurements: the data were obtained by analyzing samples of cloaca swabs, internal organs and meat of different types of chicken. The prevalence of ESBL *E. coli* and antimicrobial resistance was obtained by performing culture and antibiotics sensitivity testing of the isolates.

Sample collections: the samples of cloaca swabs and internal organs of each sampled local chicken, broiler and layer chicken were randomly collected in the market. Samples of meat from frozen imported broiler chicken from outside the Tanzania and local produced fresh broiler chicken were collected from retail meat shops. Standardized methods for sample collection and transportation procedures were employed. The samples were placed in Amie's Swab transport media and stored in a cool box before being taken to the laboratory in the maximum recovery diluent transport media. The samples were transported in cold boxes and kept at 2-8°C until ready for processing as described in the standard operating procedures [19].

Sample size: the sample size was determined using the single proportion formula: $\mathbf{n = Z^2Pq/d^2}$ where; (n) was the required sample size, Z = Z value for a given confidence level, P = expected prevalence, q = (1 - p) and d = allowable error of estimation, a confidence level was assumed to be 95% with an allowable error of 5%; thus, Z was

1.96. A prevalence of 50% was used in the calculation, which resulted in n = 384 as the minimum sample size of the chicken.

Laboratory analysis

A 25g of fresh locally produced and imported frozen chicken meat were collected. Also, cloaca swabs and internal organs of local, broilers and layer chicken were collected and immediately stored in ice cool boxes. All collected samples were transferred to the State University of Zanzibar Microbiological Laboratory on the same day for processing. Frozen imported chicken meat was thawed at 4°C before processing. Laboratory analysis was carried out according to Russel *et al.* 1997 [20].

Each sample thoroughly mixed with 225mls of Buffered Peptone Water (BPW) samples were incubated in plastic bags overnight for 18-24hrs at 37°C aerobically. The samples were then sub cultured on selective MacConkey Agar media supplemented with cefotaxime (Oxoid, England) incubated at 35-37°C for 18-24 hours. On MacConkey cefotaxime plate, presumptive ESBL *E. coli* colonies were purple or red color and were sub-cultured by streaking 3 different ESBL selective MacConkey agar plate and then incubated the plates at 37°C for 18 to 22 hours. Confirmation *E. coli* was done by convectional biochemical test strip [21].

ESBL producing *E. coli* were phenotypically confirmed by using a Double Disc Synergy Test (DDST) by cefotaxime (CX-30µg alone and cefotaxime/clavulanic acid CXT/CLA-30µg/10µg) and ceftazidime (CFZ-30µg alone and ceftazidime/clavulanic acid 10µg as recommended by Clinical & Laboratory Standards Institute (CLSI) guidelines [22]. The *E. coli* isolate was considered an ESBL producer when an increase in zone of the diameter of ≥5mm in the zone of inhibitor for ceftazidime + clavulanic acid compared to ceftazidime alone was observed as ESBL producing *E. coli* according to CLSI guidelines [22]. For quality assurance, the reference strain of *E. coli* (ATCC 26122) and *Klebsiella* (ATCC 75674) were used as positive and negative control for culture and susceptibility tests as well as for the detection of ESBL *E. coli*.

Antimicrobial susceptibility testing of ESBL producing *E. coli* isolates was performed on Muller-Hinton agar plate, using the Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines [22]. A list of 11 antibiotics of 5 classes (Oxoid, England), were tested. For quality control *E. coli* (ATCC 26122) was used. Following aerobic incubation at 37°C for 18 to 24 hours, according to CLSI guidelines. The results were considered valid when the diameter of the inhibition zone of the control *E. coli* (ATCC 26122) strain were within the performance range. Resistant and intermediate resistant isolates were collectively referred to as non-susceptible, as described [15]. Isolates were considered as Multidrug Resistant (MDR) when found non-susceptible to at least one agent in three or more antimicrobial different classes of antimicrobial agents, excluding the broad-spectrum penicillin without a β-lactamase inhibitor.

In this study the MAR indexes were calculated for each *E. coli* isolate using the formula: **MAR = a/b** where a was the number of antibiotics that the isolate was resistant to while b was the total number of antibiotics that the isolates were subjected to.

Data analysis: the data were analysed using Statistical Package for Social Science version 21 (Chicago Inc). The frequency, mean, median and standard deviation were used. The differences in the prevalence of isolates and antibiotics resistance between types of chicken were compared using the Chi-square test and Fischer's exact test. This was established based on those variables with p-value of 0.05 or less. Variables with $p<0.05$ were considered statistically significant.

Quality control: to ensure validity and reliability of the results, quality control measures were taken by using validated laboratory protocol for sample analysis. All laboratory procedures were done in accordance with standard operating procedures and CLSI guidelines. The reference strain of *E. coli* (ATCC 26122) and *Klebsiella* (ATCC 75674) were used in quality control for culture and susceptibility tests as well as for the detection of ESBL.

Results

Enrolled samples were 520, comprising of 395(76.0%) cloaca and internal organs from broilers, layers and local indigenous chicken and 125(24.0%) meat chicken. Overall, we sampled 150(37.9%) broiler chicken, (88(58.7%)) cloaca swab and 62(41.3%) chicken internal organs), 150(37.9%) layer chicken, (88(58.7%) cloaca swab and 62(41.3%) internal organs and 95(24.1%) local indigenous chicken, (60(63.2%) cloaca and 35(38.8%) internal organs. Among the 125 frozen chicken meat samples, 65(52%) were imported frozen chicken and 60(48%) were locally produced fresh chicken.

Prevalence of ESBL *E. coli* isolated in the chicken cloaca and internal organs

Out of the 395 samples, 261(73%) had *E. coli* isolates whereas 15(38.5%) were for broiler chicken and in cloaca swabs and 3(7.0%) of chicken internal organs. For the 150-layer chicken, 9(23.1%) had *E. coli* isolates in cloaca swabs and in 6(15.4%) from internal organs. Local indigenous chicken was 5(12.8%) in cloaca swab and 1(2.6%) internal organ (Table 1). Among the 261 samples, 39(14.9%) isolates were confirmed as ESBL producing *E. coli*. The overall prevalence of ESBL producing *E. coli* from all types of chicken samples by phenotypic characterization was 39(14.9%). Highest prevalence of ESBL producing *E. coli* were reported in broiler chicken at 18(46.2%) followed by 15(38.5%) among layer chicken and 6(15.4%) local indigenous chicken, respectively.

Distribution of ESBL *E. coli* isolated in the frozen chicken meat samples

Among the 125 chicken meat samples, 73(58.4%) were positive for *E. coli*. Out of the 73 isolates, 45(61.6%) of imported frozen chicken meat were positive for *E. coli* and 10(35.7%) were confirmed ESBL producing *E. coli*. A total of 28(38.4%) isolates from fresh chicken meat of local produced chicken were positive for *E. coli* and 18(64.3%) were confirmed as ESBL producing *E. coli* (Table 2).

Antimicrobial resistance profile of ESBL *E. coli* isolated in different types of chicken

In this study, all ESBL *E. coli* strains showed resistance ranging from 33.3% to 100% to the study antibiotics, namely: tetracycline 38(97.6%), ampicillin 36(96.3%), sulfamethoxazole/trimethoprim 31(79.2%), norfloxacin 28(71.0%), ceftriaxone 28(71.0%), nalidixic acid 28(69.4%), and cefotaxime 24(62.9%). In addition, 22(54.8%) isolates showed resistance to gentamycin and kanamycin. However, all isolates showed full susceptibility (100%) to amoxicillin-clavulanate and meropenem drugs (Table 3). The overall resistance of the ESBL producing *E. coli* isolated from broiler chicken to the study antibiotics ranged between 42.8% to 100% among layer chicken and 33.3% to 88.9% in locally produced chicken.

Multiple antibiotics resistant index

All isolates were resistant to 9 antimicrobial agents out of the 11 antimicrobial agents tested. The Multiple Antibiotics Resistance Index (MAR) index of ESBL ranged from, 0.1 to 0.8. In this study the lowest MAR index value of ESBL *E. coli* was 0.1 to two or more antimicrobial agents reported and (3/11) for TE, AMP and KAN. The highest MAR value of 0.8 for (9/11) was seen to combined antimicrobial agents, TET, AMP, SXT, NOR, NAL, GN, KAN, CRO, CXT; TE, AMP, KAN, NOR, NAL, GN, KAN; TE, AMP, SXT, CRO CXT, GN; TE, AMP, SXT, NAL, CXT, NAL; TE, AMP, NOR, SXT, GN; TE, AMP, SXT, CXT and TE, AMP, KAN. From this pattern,

TET, AMP, SXT, NOR, NAL, GN, KAN, CRO, CXT was the most frequent pattern of 0.8 indicated by 9/11 MAR value while the least frequent pattern was 0.1 indicated by (3/11) (Table 4).

Discussion

The *Extended Spectrum Beta Lactamase* (ESBL) producing *E. coli* is the common reported zoonotic food borne pathogen [23]. Several studies reported that chicken is an important reservoir of ESBL *E. coli* which is transmitted to human [2,4]. In the present study we aimed to investigate the prevalence of Multidrug Resistant (MDR) ESBL producing *E. coli* in live chicken and chicken products in Zanzibar. The overall prevalence of ESBL producing *E. coli* from all types of chicken samples was 39(14.9%). The reported prevalence is lower than compared to earlier studies conducted in Egypt where they reported a prevalence of 25.3% [14] and 49.9% in Tanzania Mainland [15]. In the current study, higher prevalence of ESBL *E. coli* was observed in samples of cloaca swab and internal organs from broiler chicken than in other chickens. This is in agreement with the findings reported in the study conducted in Tanzania by Mgaya *et al.* (2018) [11].

In the present study, high prevalence of ESBL *E. coli* was observed in fresh meat samples from locally produced chicken compare to that in frozen meat of imported chicken. This is consistent with the findings reported from a study conducted by Apostolakos *et al.* (2019) in Italy [24]. The difference in prevalence might be due to difference in knowledge and awareness of biosecurity measures and hygiene practices shown by the farmers in the farming sites. Moreover, ESBL producing *E. coli* present in chicken and the products of both local improved chicken as well as the meat of fresh produced and imported frozen meat in Zanzibar. In the present study, the overall prevalence of ESBL *E. coli* in chicken was 29 (14.9%). The prevalence observed in the present study was higher than the findings of the previous study by Mgaya *et al.* (2021) conducted in Tanzania Mainland [25]. However, the prevalence reported in the current study is lower than the findings of the studies in Nigeria where they reported a prevalence of 60.3% [26] and 29% in Ghana [27].

The finding of the present study shows that ESBL producing *E. coli* isolates were more resistant to more than one antibiotic, especially tetracycline, penicillin (ampicillin), sulphonamide (sulfamethoxazole/trimethoprim), quinolone (norfloxacin and nalidixic acid). This may have been contributed by the high frequency of misuse of antibiotics due to their broad-spectrum nature, which are also easily available in veterinary pharmaceutical outlets with little restriction. The discriminate use of the antibiotics by chicken farmers for prophylaxis and therapeutic purposes promote the occurrence of resistant genes thus escalating the magnitude of antimicrobial resistance [28]. Susceptibility of the isolates were observed to amoxicillin-clavulanate and carbapenem drugs and none of the isolates indicated resistance to meropenem. This implies that such drugs are more effective in the treatment of ESBL *E. coli* associated infections in chicken. In addition to the stability of the carbapenem drugs against activity of ESBLs enzyme produced many enterobacteriaceae bacteria [29]. Our findings are consistent to those of the study done by Mehdi *et al.* (2018) in Canada [9]. However, our findings are in contrast with the study conducted in which reported a low level of antimicrobial resistance [30]. In the current study, the resistance to third generation cephalosporin drugs (ceftriaxone and cefotaxime) was quite high. This is supported by the study by Park (2014) in Korea [31]. The observed high resistance might be explained by its β-lactam nature which makes them susceptible to ESBL enzymes, which hydrolize the beta-lactam ring of the antibiotics including cephalosporin drugs [32]. Our study reported high rate of MDR to all tested classes of antibiotics. This is consistent with other previous studies by Bergšpica *et al.* in Europe and Li *et al.* (2022) in China [6,33]. The MDR shown among the bacteria in the present study is due to the presence of multiple drug efflux pump which can pump out more than one antibiotic.

Overall, all isolates were resistant to 9 antimicrobial agents out of the 11 antimicrobial agents tested. In the current study, the MAR index of ESBL producing *E. coli* ranged from, 0.1 to 0.8. The MAR index reported in this study is supported by earlier studies conducted in Nigeria [17] and in India [34]. The highest MAR index 0.8, indicated possible drug interaction between the reported resistant antimicrobial agents. The reported MAR index greater than 0.2 implies a high contamination risk where frequent antibiotics were used [17]. The frequency and overuse of antibiotics gives rise to MDR in associated bacteria that acquire antibiotic-resistant genes [32,34]. This is through the production of the extended-spectrum β-lactamases causing infectious diseases which are difficult to treat [8]. The resistant gene may be transferred between bacteria from animals to human beings giving rise to zoonotic antibiotic resistance [14].

Conclusion

The findings in the current study shows that ESBL *E. coli* were prevalent in chicken and their products. The ESBL producing *E. coli* was more prevalent in broiler chicken compared to the meat of fresh locally produced broiler chicken. A high level of antimicrobial resistance to ESBL producing *E. coli* against commonly used antibiotics for treatment of chicken infections was observed. Carbapenem and β-lactam inhibitor was more effective in the treatment against ESBL producing *E. coli* infections. There is a need for the government authorities to enforce regulations for prescription of antibiotics to treat bacterial infections in chicken production. There is also a need to establish antimicrobial use stewardship in livestock and farmers to comply with biosecurity measures in chicken farming.

What is known about this topic

- *Antimicrobial resistance due to emergency of ESBL E. coli is a global health threat;*
- *Chicken is a known reservoir of ESBL producing E. coli;*
- *Over and irrational use of antibiotics in food animal production are a primary source of developing resistant bacteria.*

What this study adds

- *The study found that chicken produced in Zanzibar are contaminated with ESBL producing E. coli;*
- *Fresh meat of locally produced chicken is highly contaminated with ESBL producing E. coli compared to frozen meat of imported chicken;*
- *The study reported high level of multidrug resistant ESBL producing E. coli to common antibiotics used in the treatment of poultry infections.*

Competing interests

The authors declare no competing interests.

Authors' contributions

Muhiddin Omar, Andrew Martin Kilale, Angaza Amos Gimbi, Huba Khamis Rashid, Mayassa Salum Ally contributed to the study design and wrote the manuscript. Muhiddin Omar, Huba Khamis Rashid, collected data. Muhiddin Omar, Andrew Martin Kilale, Angaza Amos Gimbi, Mayassa Salum Ally, reviewed and analyzed the data. Muhiddin Omar, Mayassa Salum Ally, Huba Khamis Rashid, prepared the tables. All the authors read and approved the final manuscript.

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Tables

Table 1: prevalence of ESBL *E. coli* and Non ESBL *E. coli* isolated in chicken cloaca and internal organs

Table 2: proportion of ESBL *E. coli* isolated in fresh and frozen meat of imported and local produced fresh chicken in Zanzibar

Table 3: antimicrobial resistance profile of ESBL *E. coli* isolated from chicken samples in Zanzibar

Table 4: overall resistance pattern observed in MDR ESBL *E. coli* isolated in chicken in Zanzibar (n= 39)

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Table 1: prevalence of ESBL *E. coli* and Non ESBL *E. coli* isolated in chicken cloaca and internal organs					
Chicken type	**Sample type**	**Total samples**	**Positive**	**ESBL *E. coli* n (%)**	**Non ESBL *E. coli* n (%)**
Broilers	Cloaca swab	88(58.7)	72(27.6)	15(38.5)	57(25.7)
	Internal organs	62(41.3)	37(14.2)	3(7.0)	34(15.3)
Sub-total		150(37.9)	109(41.8)	18(46.2)	91(40.9)
Layers	Cloaca swab	88(58.7)	58(22.2)	9(23.1)	49(22.1)
	Internal organs	62(41.3)	26(11.1)	6(15.4)	20(9.0)
Sub-total		150(37.9)	84(32.1)	15(38.5)	69(31.1)
Local	Cloaca swab	60(63.2)	42(16)	5(12.8)	37(16.7)
	Internal organs	35(38.8)	26(9.9)	1(2.6)	25(11.3)
Sub-total		95(24.1)	68(26.1)	6 (15.4)	62(27.9)
TOTAL		395	261(7.3)	39(14.9)	222(85.1)

Table 2: proportion of ESBL *E. coli* isolated in fresh and frozen meat of imported and local produced fresh chicken in Zanzibar				
Source of the sample	**Sample type**	**Number of samples n (%)**	**Positive samples for *E. coli* n (%)**	**ESBL *E. coli* n (%)**
Imported frozen chicken	Meat	65(52.0)	28(38.4)	10(35.7)
Local produced fresh broiler chicken	Meat	60(48.0)	45(61.6)	18(64.3)
Total		**125**	**73(58.4)**	**28(38.4)**

Table 3: antimicrobial resistance profile of ESBL *E. coli* isolated from chicken samples in Zanzibar							
Drug class	**Antibiotics**	**Abbreviations**	**Concentration (µg)**	**Percentage of resistant isolates (N = 39)**			**Average resistance n(%)**
				Local chicken n(%) N=9	**Layer n(%) N=14**	**Broiler n(%) N=16**	
Tetracycline	Tetracycline	TE	30	8(88.9)	13(92.9)	16(100)	38(97.6)
Penicillin	Ampicillin	AMP	30	8(88.9)	14(100)	16(100)	36(96.3)
Sulphonamide	Sulfamethoxazole	SXT	(1.25/23.75)	7(77.8)	11(78.6)	13(81.3)	31(79.2)
Quinolones	Norfloxacin	NOR	10	6(66.7)	10(71.4)	12(75.0)	28(71.0)
	Nalidixic Acid	NAL	30	5(55.5)	10(71.4)	13(81.3)	28(69.4)
Aminoglycoside	Gentamycin	GN	30	4(44.4)	8(57.4)	10(62.5)	22(54.8)
	Kanamycin	KAN	30	3(33.3)	6(42.8)	8(50.0)	18(44.4)
β-lactam inhibitor	Amoxicillin-clavulanate	AMCL	30	0	0	0	0
Cephalosporin	Ceftriaxone	CXT	30	6(66.7	10(71.4)	12(75.0)	28(71.0)
	Cefotaxime	CAZ	30	5(55.6)	9(64.3)	10(68.7)	24(62.9)
Carbapenem	Meropenem	MRP	10	0	0	0	0

Table 4: overall resistance pattern observed in MDR ESBL *E. coli* isolated in chicken in Zanzibar (n= 39)			
Antimicrobial resistance pattern	**Resistant antibiotics**	**Resistant isolates n(%)**	**MAR INDEX**
TET, AMP, SXT, NOR, NAL, GN, KAN, CRO, CXT	9	12(30.7)	0.7
TE, AMP, KAN, NOR, NAL, GN, KAN, CRO	8	9(23.1)	0.6
TE, AMP, SXT, CRO CXT, GN, NOR	7	5(12.8)	0.5
TE, AMP, SXT, NAL, CXT, NAL	6	6(15.4)	0.4
TE, AMP, NOR, SXT, GN	5	4(10.3)	0.3
TE, AMP, SXT, CXT	4	3(7.7)	0.2
TE, AMP, KAN	3	1(2.5)	0.1
Abbreviations: TET = Tetracycline, AMP = Ampicillin = SXT = Sulfamethoxazole/Trimethoprim, NOR = Norfloxacin, NAL = Nalidixic acid, GN = Gentamycin, KAN = Kanamycin, CRO = Ceftriaxone, CXT = Cefotaxime			