Antimicrobial Resistance of Escherichia coli and Salmonella isolated from Raw Retail Broiler Chicken Carcasses in Zambia

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Abstract

Background Antimicrobial resistance (AMR) of foodborne pathogens is of public health concern, especially in developing countries such as Zambia. This study was undertaken to determine the antimicrobial resistance profiles of *Escherichia coli* (*E. coli*) and *Salmonella* isolated from raw retail broiler chicken carcasses purchased from open and supermarkets in Zambia.

Results A total of 189 *E. coli* and five *Salmonella* isolates were isolated. Identification and confirmation of the isolates were done using Analytical Profile Index (API 20E) (Biomerieux®) and 16S rRNA sequencing. Antimicrobial susceptibility tests (AST) were performed using the Kirby Bauer disk diffusion technique using a panel of 10 antibiotics. Multiplex PCR was used to determine the presence of three target genes encoding for resistance: *tet*A, *Sul*1 and $bla_{\text{CTX-M}}$. WHONET 2018 software was used to analyse AST results. The *E. coli* isolates were mostly resistant to tetracycline (79.4%), ampicillin (51.9%), and trimethoprim/sulfamethoxazole (49.7%). Two of the five *Salmonella* isolates were resistant to at least one antibiotic. Forty- seven (45.2%) of the 104 isolates that were screened for the presence of the resistant genes possessed at least one of the targeted resistance genes.

Conclusion This study has demonstrated the presence of AMR *E. coli* and *Salmonella* on raw retail broiler chicken carcasses from open and supermarkets, which is of public health concern.

Background

Poultry meat forms an integral part of the diet, especially in developing countries. It is popular because it is a cheaper source of protein and easy to produce compared to other meat products (Musaba and Mseteka, 2014). However, the high demand for poultry meat puts a strain on producers, who have to meet the ever-growing demand and also realise profits in a competitive market environment (Ahuja and Sen, 2007). One of the strategies producers often resort to is the use of antibiotics to prevent and treat diseases of poultry to optimize growth (Apata, 2009).

Antibiotics are also used for growth promotion. However, if they are misused, they can lead to the development of resistance in bacteria found in chickens. In the absence of a national surveillance system on the use of antibiotics, it is difficult to know whether they are being used appropriately (WHO Global Report, 2014).

In monitoring development of antimicrobial resistance (AMR) in bacteria, *Escherichia coli* (*E. coli*) is commonly used because it is part of the gut microbiota and is a reservoir for AMR genes (Van Schaik, 2015; Yassin *et al.*, 2017). AMR occurs when bacteria, viruses, fungi and parasites no longer respond to the antimicrobials designed to kill them (Centers for Disease Control and Prevention, 2018). Despite *E. coli* being an innocuous resident of the digestive system, it can also be pathogenic and cause severe intestinal and extra-intestinal diseases (Diarrassouba *et al.*, 2007). Recent studies showed that *E. coli* is involved in 48 million reported cases of foodborne illness worldwide (CDC, 2016). Furthermore, among the foodborne bacterial enteropathogens, *E. coli, Salmonella* sp. and *Campylobacter* sp. are responsible

for most of the diarrhoeal diseases that affect millions of people annually, sometimes with fatal outcomes (WHO, 2015).

Besides, non-typhoidal *Salmonella* species are responsible for causing gastroenteritis and bacteremia, which can eventually lead to secondary infection, especially in immune-compromised individuals such as patients with malignancy, human immunodeficiency virus, diabetes, and those receiving medication for anti-inflammatory diseases (Gordon, 2008).

E. coli and *Salmonella* sp. infections can be treated using relevant antibiotics. However, there is accumulating evidence of the consequences of AMR including, the reduction in the efficacy of treatment with first-line drugs and limited choices after microbiological diagnosis (Clarke *et al*., 2012; Mshana *et al*, 2013).

In Zambia, recent findings showed that *Salmonella* sp. and *E. coli* (18% and 25.5%, respectively) were among the most detected organisms causing bacterial diarrhoeal disease in children between the ages of 0-59 months at the University Teaching Hospital (Chiyangi *et al*., 2017). In this age category, most of the diarrhoeal cases are more likely to be caused by foodborne pathogens especially in developing countries where food safety problems and poor hygiene and sanitation are recurrent (Byarugaba, 2004; Thapar and Sanderson, 2004).

Further, several reports from different countries elucidate a growing concern of AMR in foodborne pathogens which have a negative impact on public health (Clarke *et al*., 2012; Mshana *et al*, 2013; Mainda *et al*., 2015). However, there is a paucity of information on AMR occurrence on foodborne pathogens in Zambia. Furthermore, in a growing industry of broiler chicken production and consumption in Zambia, the misuse of antimicrobials for growth promotion, prevention and treatment of diseases could contribute to the occurrence of AMR in foodborne pathogens; yet few studies have been conducted to ascertain the magnitude of the problem and generate information that could address the AMR problem.

This study, therefore, aimed at characterizing the phenotypes and genotypes of antimicrobial-resistant *E. coli* and *Salmonella* on raw retail broiler chicken carcasses in Zambia.

Results

Descriptive statistics

A total of 332 raw retail broiler chicken carcasses were sampled from both supermarkets (154) and open markets (178), from which 189 (56.9%) and 05 (1.5%) E. coli and Salmonella, respectively were isolated and identified. The two pathogens of interest were mostly isolated from supermarkets (Table 2).

Antimicrobial susceptibility profiles

E. coli isolates from raw retail broiler chicken carcasses obtained from open markets had a higher

occurrence of resistance of 91.7% (n=88) to at least one antimicrobial agent in the AST testing, while those from supermarkets recorded 83.9% (n=78). The overall resistance of E. coli isolates to at least one antimicrobial agent for both the open markets and supermarkets was 88% (n=166). However, 102 E. coli and the 02 Salmonella isolates were completely resistant to at least one of the antimicrobials used. The E. coli isolates were resistant to tetracycline, trimethoprim-sulfamethoxazole and ampicillin with 79.4% (n=150 isolates), 51.9% (n=98 isolates) and 49.7% (n=94 isolates) resistance, respectively (Table 3) while only ampicillin and tetracycline recorded resistance among the Salmonella isolates (Table 4).
None of the Salmonella isolates was Multidrug-resistant (MDR). MDR was defined as acquired nonsusceptibility to at least one agent in three or more antimicrobial categories (Basak et al, 2016) However, 36.5% (n=69) of the E. coli isolates were MDR and 26 different patterns were observed (Table 5). The most common MDR patterns that were observed among the E. coli isolates were CHL-TCY-AMP-SXT (15.9%), CTX –TCY-AMP-SXT (10.1%), CIP-TCY-AMP-SXT (8.7%).

Determination of Antimicrobial resistance genes

Due to limited resources, only 102 E. coli and 2 Salmonella isolates that were completely resistant to at least one of the antimicrobials used were analyzed for the presence of resistance genes. The presence of at least one of the targeted genes was detected in 45.2% (n=47) of the E. coli isolates. The Salmonella isolates did not show the presence of the targeted genes. The beta-lactamase-encoding genes (blaCTX-M) were the most commonly detected among the 47 E. coli isolates.

Discussion

This study isolated *E. coli* and *Salmonella* from raw retail broiler chicken carcasses traded in supermarkets and open markets in selected districts in Zambia. It further characterized the phenotypes and genotypes of AMR phenotypes and genotypes of *E. coli* and *Salmonella* isolates.

It was observed that the recovery rate of *E. coli* and *Salmonella* sp was higher in samples from supermarkets (60.4% and 2.6%, respectively) as compared to those from open markets. These findings are different from those of a similar study conducted in Thailand where authors reported a low recovery rate of 25% and 2% for *E. coli* and *Salmonella,* respectively (Vindigni *et al.*, 2007). The disparity of findings could be attributed to a difference in processing methods and commercial production techniques which are highly automated in Thailand as compared to the Zambian settings where the manual system is often used hence exposing the carcasses to contamination.

The overall contamination level of *Salmonella* sp in all samples was low (1.5%). This was also observed in some studies done at the retail level (Cosby *et al.*, 2015; Mpundu *et al.*, 2019). The low contamination rate could be attributed to the intermittent shading of *Salmonella* in poultry (Van Immerseel *et al.*, 2004).

E. coli and *Salmonella* sp. isolated from this study showed a high level of antibiotic resistance including resistance to multiple antibiotics. *E. coli* isolates were resistant to tetracycline (79.4%), ampicillin (51.9%) and trimethoprim/sulfamethoxazole (49.7%). This has also been observed in other parts of the world

(Talebiyan *et al*., 2014) and could be attributed to the use of these antibiotics as growth promotors and for prevention of infection rather than for therapeutic purposes only (Agyare *et al*., 2019). *Salmonella* isolates were resistant to ampicillin (60.0%) and tetracycline (40.0%). Similar resistance profiles for *Salmonella* isolated from food animals was reported by Johnson et al. (2005) in a study conducted in Canada (Johnson *et al*, 2005).

The high resistance to tetracyclines, sulfonamides and beta-lactam antibiotics could be attributed to the abuse of antibiotics in both livestock and humans, especially among small poultry producers. In Zambia, there is poor regulation of veterinary drugs and antibiotics, whereby farmers can purchase antibiotics over the counter without a prescription (Mainda, 2016; Manyi-Loh *et al*., 2018). Further, the poor hygienic processing methods that are employed by small and medium-scale producers may facilitate the contamination of the carcasses with AMR organisms. The handling of the carcasses during slaughter, rinsing, transportation and sale may also introduce resistant organisms from humans and the environment into the carcasses. Raw retail broiler chicken carcasses that originate from commercial abattoirs, however, may get contaminated mostly from the abattoir bench surfaces and intestines of the broilers during processing (Voidarou *et al*., 2011).

Multidrug resistance was also reported among 69 (36.5%) of the 189 *E. coli* isolates in this study. These findings are similar to other studies on AMR in both poultry and humans (van den Bogaard, 2001; Chiyangi *et al*., 2017). The high resistance in poultry, especially, can be attributed to the use of antibiotics for preventive purposes by small-scale poultry farmers between weeks 4 and 6 of production to prevent high mortality rates due to colibacillosis in broilers (Nolan *et al*., 2017). It has also been documented that tetracycline and sulfadimidine are among the commonly used antibiotics for therapy, especially at small-scale production (Mainda *et al*., 2015). Further, farmers tend to self-prescribe these drugs whenever they have a disease situation when raising the birds (Guetiya Wadoum *et al*., 2016; Xu *et al*., 2020). Such misuse of antibiotics in livestock production has been reported to cause AMR (Lowe, 1982; Ngoma *et al*., 1993; Koluman and Dikici, 2013; Kalonda *et al*., 2015; Ayukekbong *et al*, 2017).

Of the three resistant genes that were targeted, the beta-lactams gene ($bla_{\text{CTX-M}}$ gene) was the most detected in this study. *E. coli* strains harbouring these genes have been detected in both healthy and sick poultry (Girlich *et al*., 2007) and are an indication of Extended-Spectrum Beta-Lactamase (ESBL) producing *E. coli*. The beta-lactam gene of interest that was targeted is similar to the one that was found by other researchers who did similar studies (Chishimba *et al*., 2016; Ramachandran *et al*, 2017). Though the other two genes for resistance to sulfonamides and tetracyclines (*sul*1 and *tet*A) were also detected, the detection rates were not as high as that of the beta-lactamase-encoding gene of interest. These discrepancies could be attributed to differences in target sequences of the resistance genes that were being targeted or other genes other than the ones chosen for this study.

Although the scope of molecular analysis was limited in this study the indication is that most phenotypic resistance that was observed in *E. coli* and *Salmonella* isolates were encoded by the genes that encodes tetracyclines, beta lactams and sulphur based antibiotics. These antibiotics have been reported to be the

most commonly used in Zambia and are relatively preferred because of the affordable price by most livestock (Mainda *et al.*, 2015).

Conclusion

This study has demonstrated the presence of AMR *E. coli* and *Salmonella* on raw retail broiler chicken carcasses in selected districts in Zambia. It has also shown the presence of MDR and ESBL producing *E. coli* that are of public health concern, if acquired by humans. The *E. coli* and *Salmonella* isolates were most resistant to tetracyclines, beta-lactams, sulfonamide and fluoroquinolone antibiotics. This could be attributed to the misuse of antibiotics at both commercial and small-scale levels of production. There is a need to regulate the use of antibiotics during broiler chicken production. Further, more molecular work that can give a complete understanding of the actual genes conferring resistance in Zambia will be beneficial to guide AMR policy formulation.

Methods

Study Design

A cross-sectional study was conducted between August 2017 and May 2018 in seven districts in Zambia that included Lusaka, Chilanga, Chongwe, Kafue, Choma, Kabwe and Kitwe. Choma, Kabwe, Kitwe and Lusaka districts were purposely selected because as provincial headquarters, they are retail destinations for many poultry products from other districts while Chilanga, Chongwe and Kafue districts were included due to their proximity to Lusaka, the capital city. The primary sampling units were the markets (broadly classified as Open markets and Supermarkets) and the secondary sampling units were the raw retail broiler chicken carcasses. An open market was defined as an unrestricted market, not housed in a building, where food products are often sold exposed, while a supermarket was defined as a restricted market, housed in a closed building with modernized facilities (Bumbangi *et al.*, 2016).

Proportion stratified random sampling was employed where Open markets and Supermarkets were the strata. At the time of the study, information collected from the respective City Councils revealed that there were 47 supermarkets and 33 open markets in Lusaka province, five open markets and four supermarkets in Choma, seven open markets and eight supermarkets in Kitwe, nine open markets and six supermarkets in Kabwe, three open markets and two supermarkets in Kafue, three open markets and one supermarket in Chilanga, and one open market and no supermarket in Chongwe. This formed the sampling frame from which a study population was drawn.

Sample size calculation

The sample size for estimation of a single proportion was calculated using Epi tools software (www.epitools.ausvet.com) based on the following assumptions: prevalence of AMR *E. coli* on raw retail broiler chicken carcasses = 25% (Vindigni *et al.*, 2007); confidence level = 95%; level of precision = 5%.

Using the above assumptions, the minimum sample size calculated was 289 and the distribution of sampling sites is as outlined in Table 1.

Sampling from open markets

From the open markets within Lusaka district that were sampled (n=22), two shops trading in raw retail broiler chicken carcasses at each market were selected and from each shop two raw retail broiler chicken carcasses purchased. Furthermore, at the market where open stands (display tables) instead of shops trading in dressed broiler chickens were available, one sample from each stand was collected. For other districts, considering the limited number of markets that traded in raw retail broiler chicken carcasses, all of them were included in the study and at least two samples from each market were collected bringing the total number of samples from open markets to 178 (Table 1).

Sampling from the Supermarket

From each supermarket in all districts, a maximum of four different brands of raw retail broiler chicken carcasses was sampled (one of each brand), though some sampling sites had less than the maximum number. Therefore, the number of broiler carcasses from supermarkets in this study was 154 (Table 1).

Upon purchase, all samples were transported in a cooler box containing ice packs to the laboratory and processed within 8 hours.

Laboratory Analysis

Laboratory isolation included a whole carcass rinse in buffered peptone water (Oxoid) (Figure S1a-c), pre-enrichment of an aliquot of the rinsate and subsequent incubation at 37^{O}C overnight. Processing of pre-enriched broths was undertaken in the Public Health Laboratory, School of Veterinary Medicine. The Carcass Rinse technique outlined by the USDA Food Safety and Inspection Service was used with a few modifications (D'Aoust et al, 1982). Whole chicken carcasses were aseptically placed in sterile 3 litre ziplock bags. 450mL of sterile buffered peptone water (Oxoid UK) was poured into each of the bags containing the carcasses. The open ends of the bags were then twisted to hold the bags closed and the entire carcass was rinsed using a repeated rocking motion 30 times. The bags were then opened and the carcasses removed. The rinsate was then collected in sterile containers by puncturing the "v"-end of the bags and used for further analysis.

The methods proposed by the Food and Drug Administration's Bacteriological Analytical Manual (U.S. Food and Drug Administration, 2001) were used with a few modifications for the isolation of *Salmonella* and *E. coli*. During the carcass rinse technique, 450mL of sterile buffered peptone water was also poured into an empty bag that did not contain a carcass to act as a control. The rinsate was incubated overnight and later streaked onto MacConkey agar plate (Oxoid, UK) to ensure that the batch of bags was sterile and that the organisms isolated were indeed from the chicken carcasses and not the bags used for rinsing. 10µL of the incubated broth was then transferred to MacConkey agar (Oxoid UK) and resulting

colonies were gram stained for detection of Gram-negative short rods, which were subsequently sub-cultured onto Eosin Methylene Blue (EMB) agar (Oxoid UK). Colonies that showed a metallic green sheen (Figure S2) were subjected to biochemical tests using Analytical Profile Index (API 20E) (Biomerieux®) for identification for *E. coli* isolates. 1ml of the incubated pre-enrichment broth was also transferred to Rappaport Vassiliadis (Oxoid UK) and later subcultured on Xylose-Lysine Deoxycholate agar (Oxoid UK). Pink and black colonies on XLD agar (Figure S3) were then Gram-stained and subjected to biochemical tests for identification of *Salmonella* using API 20E. Further confirmation of the isolates was done using 16S rRNA sequencing (Weisburg *et al*., 1991).

The Kirby-Bauer disk diffusion technique for AST was used on all confirmed *Salmonella* and *E. coli* isolates using a panel of 9 different antibiotics (Kirby-Bauer, 1961). The isolates were prepared by sub-culturing onto Blood agar (Oxoid UK) overnight at 37^{O}C. One or two colonies were then suspended in 4mL of 0.9% sodium chloride solution and their turbidity compared to that of a 0.5 McFarland's turbidity standard. An inoculum of the suspension was then spread on two Müeller Hinton agar (4 ml thickness) plates (Oxoid UK) until the entire surfaces of the plates were covered. 5 different antibiotic wafers from the 9 chosen for the study were then placed on the surface of each of 2 plates using the applicator (Oxoid). Two plates were used for each isolate to accommodate the 9 antibiotics. The antibiotics that were used were amoxicillin-clavulanic acid (30µg), ampicillin (10µg), cefotaxime (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), imipenem (10µg), nalidixic acid (30µg), tetracycline (30µg) and trimethoprim-sulfamethoxazole (25µg). The list of antibiotics was prioritized based on the most frequently used in the poultry industry in Zambia and also based on the priority list by the WHO and OIE of critical antibiotics (Oie, 2007; WHO, 2017). The plates were then incubated at 37^{O}C for 24hrs and the diameters of the zones of inhibition entered and analyzed in WHONET 2018 software. An isolate was considered to be completely resistant to an antimicrobial when it had no zone of inhibition around the antimicrobial disc after the incubation (Kirby-Bauer, 1961). The interpretation of AST results was based on the Clinical and laboratory standards institute (CLSI) guidelines 2018 (Replaces *et al*., 2018)

Isolates which showed resistance to tetracyclines, sulphonamides and beta-lactam antibiotics were then forwarded for molecular analysis that involved extraction of DNA and checking for the presence of target resistance genes. The process of DNA extraction involved the suspension of a few bacterial colonies in 100µL of nuclease-free water and heating of the vials at 80^{O}C for 10 minutes. The suspension was then centrifuged at 60000G with a temperature of 4°C for 3 minutes. Multiplex polymerase chain reaction (PCR) was performed to check the presence of resistant genes of interest according to the method described by Adesiji et al. 2014. The mastermix volumes and PCR reaction were as outlined in Table S1 and Table S2 (Supplementary Material). The target genes were selected based on the antimicrobial susceptibility results. The 3 target genes were *tet*A (for tetracycline resistance), *sul*1 (for sulfonamide resistance) and $bla_{\text{CTX-M}}$ (for beta-lactam resistance) (Table S3; Figures S4**).** Every batch of samples was processed along with a positive and negative control using *E. coli* 25922 (ATCC) and *Salmonella typhimurium* 14028 (ATCC).

Abbreviations

AMR: Antimicrobial Resistance

AST: Antimicrobial Susceptibility Testing

MDR: Multi-drug Resistance

ESBL: Extended-Spectrum Beta-Lactamase

Declarations

Ethics approval and consent to participate

Ethical approval was given by ERES Converge IRB under the WHO-AGISAR project titled *Investigation of foodborne pathogens and their antimicrobial resistance in animals and humans – A pilot project for Zambia (ID: 204954).*

Consent for publication

Not applicable

Availability of Data and Materials

The datasets supporting the conclusions of this article are available within the article (Tables) and separate data files uploaded (Figures). Any additional datasets required are available from the corresponding author on request.

Competing Interests

The authors declare that there is no conflict of interest regarding this publication.

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Author's contributions

EMM was the principle investigator, GM, MM[2], GK and BNF edited the document, NP and MM[8] helped with sample collection and processing, BH and MM[4] were co-supervisors while JBM was the principle supervisor and recipient of the funds that ensured the completion of the research. All authors have read and approved the manuscript.

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Tables

Table 1: Summary distribution of samples collected by market type and district

District	No. of open markets	No. of Supermarkets	Total No. of markets	Samples from Open Markets (%)	Samples from Supermarkets (%)	Total No. of samples (%)
Chilanga	2	1	3	8 (4.5)	4 (2.6)	12 (3.6)
Chongwe	1	0	1	11 (6.2)	0 (0.0)	11 (3.3)
Kafue	1	2	3	2 (1.1)	6 (3.9)	8 (2.4)
Lusaka	22	34	56	113 (63.5)	106 (68.8)	219 (66.0)
Kitwe	7	8	15	15 (8.4)	18 (11.7)	33 (9.9)
Choma	4	2	6	14 (7.9)	7 (4.5)	21 (6.3)
Kabwe	5	3	8	15 (8.4)	13 (8.5)	28 (8.5)
Total	**42**	**50**	**92**	**178 (100.0)**	**154 (100.0)**	**332 (100.0)**

Table 2: Distribution of isolates according to market types and district

District	Open Market			Supermarket		
	No. of samples	No. of *E. coli* (%)	No. of *Salmonella* (%)	No. of samples	No. of *E. coli* (%)	No. of *Salmonella* (%)
Chilanga	8	0 (0.0)	0	4	4 (100.0)	0
Chongwe	11	4 (36.4)	0	0	0	0
Kafue	2	2 (100.0)	0	6	6 (100.0)	0
Lusaka	113	73 (64.6)	1	106	59 (55.7)	4
Kitwe	15	8 (53.3)	0	18	15 (83.3)	0
Choma	14	7 (50.0)	0	7	5 (71.4)	0
Kabwe	15	2 (13.3)	0	13	4 (30.8)	0
Total	**178**	**96 (53.9)**	**1 (0.5)**	**154**	**93 (60.4)**	**4 (2.6)**

Table 3: Resistance profiles for E. coli isolates

Antimicrobial	**Breakpoints (mm)**	**Number**	**%R**	**%I**	**%S**	**% R 95%C.I.**
Ampicillin	14 – 16	189	51.9	4.8	43.4	44.5-59.2
Amoxicillin/Clavulanic acid	14 – 17	189	6.9	5.8	87.3	3.9-11.8
Cefotaxime	23 – 25	189	16.4	6.3	77.2	11.6-22.6
Imipenem	20 – 22	189	1.1	6.3	92.6	0.2-4.2
Nalidixic acid	14 – 18	189	24.3	9.5	66.1	18.5-31.2
Ciprofloxacin	16 – 20	189	10.1	4.2	85.7	6.4-15.5
Trimethoprim/Sulfamethoxazole	11 – 15	189	49.7	0.5	49.7	42.4-57.0
Chloramphenicol	13 – 17	189	16.4	4.8	78.8	11.6-22.6
Tetracycline	12 – 14	189	79.4	2.1	18.5	72.8-84.8

Table 4: Resistance profiles for Salmonella isolates

Antimicrobial	Breakpoints (mm)	Number	%R	%I	%S	%R 95%C.I.
Ampicillin	14 – 16	5	60	0	40	17.0-92.7
Amoxicillin/Clavulanic acid	14 – 17	5	0	20	80	0.0-53.7
Cefotaxime	23 – 25	5	0	20	80	0.0-53.7
Imipenem	20 – 22	5	0	0	100	0.0-53.7
Nalidixic acid	14 – 18	5	0	20	80	0.0-53.7
Ciprofloxacin	21 – 30	5	0	20	80	0.0-53.7
Trimethoprim/Sulfamethoxazole	11 – 15	5	0	0	100	0.0-53.7
Chloramphenicol	13 – 17	5	0	0	100	0.0-53.7
Tetracycline	12 – 14	5	40	20	40	7.3-83.0

Table 5: Antimicrobial Resistance Patterns for MDR isolates.

Antimicrobial Resistance Pattern	Number of isolates with pattern (n, %)
CTX-TCY-AMP	2 (2.9%)
CHL-TCY-SXT	2 (2.9%)
CHL-TCY-AMP	3 (4.3%)
CHL-CTX-TCY	1 (1.4%)
CHL-CIP-TCY	1 (1.4%)
TCY-AMP-SXT	2 (2.9%)
IPM-TCY-AMP-SXT	1 (1.4%)
CTX-TCY-AMP-SXT	7 (10.1%)
CTX-IPM-TCY-SXT	4 (5.8%)
CIP-TCY-AMP-SXT	6 (8.7%)
CIP-IPM-AMP-SXT	1 (1.4%)
CIP-CTX-TCY-SXT	2 (2.9%)
CIP-CTX-TCY	1 (1.4%)
CHL-TCY-AMP-SXT	11 (15.9%)
CHL-CTX-AMP-SXT	1 (1.4%)
CHL-CTX-TCY-SXT	1 (1.4%)
CHL-CIP-TCY-SXT	3 (4.3%)
CTX-TCY-AMP-SXT	1 (1.4%)
CIP-IPM-TCY-AMP-SXT	1 (1.4%)
CHL-IPM-TCY-AMP	2 (2.9%)
CHL-CTX-TCY-AMP-SXT	4 (5.8%)
CHL-CIP-TCY-AMP-SXT	5 (7.2%)
CHL-CIP-IPM-TCY-AMP-SXT	1 (1.4%)
CHL-CIP-CTX-TCY-AMP-SXT	4 (5.8%)
IPM-TCY-SXT	1 (1.4%)
CTX-TCY-SXT	1 (1.4%)
Total MDR	**69 (99.2%)**

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