

Original Article

## ANTIMICROBIAL RESISTANCE PROFILES OF BACTERIA ISOLATED FROM CHICKEN DROPPINGS IN DAR ES SALAAM

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### ABSTRACT

**Objective:** To determine resistance profiles of bacteria isolated from chicken droppings.

**Methods:** It was a cross-sectional study involving collection of fresh chicken droppings from 100 chickens from 13 localities; followed by microbiological analysis using standard procedures. Multiple antibiotic resistance indices (MAR) were also determined for each of the isolated bacteria.

**Results:** A total of 188 bacteria were isolated and subjected to susceptibility testing against 9 commonly used antibiotics. All tested bacteria exhibited multiple resistance to the antibiotics with MAR rates in this order *Escherichia coli*>*Pseudomonas aeruginosa*>*Klebsiella pneumoniae*>*Staphylococcus aureus*. More than half of *P. aeruginosa* and *Salmonella typhi* isolates were resistant to Ceftriaxone and Amikacin, while 77% of *K. pneumoniae* isolates were resistant to Chloramphenicol.

**Conclusion:** High rates of antibiotic resistance were observed to clinically used antibiotics among the isolated bacteria; suggesting that chicken rearing may serve as the reservoir of antibacterial resistant bacteria transmissible to human through the food chain.

**Keywords:** Chicken feces, Bacterial isolates, Multiple antibiotic resistance index.

### INTRODUCTION

Chicken meat is the second most eaten worldwide after pork [1]. Chicken husbandry is a popular business in Tanzania and thus chicken meat is readily available in the market. During chicken slaughtering, carcasses can be contaminated with fecal matters from the chicken's intestines. Bacterial infections due to *Escherichia coli* and *Salmonella* spp have been contracted through such process or consumption of under cooked chicken meat [2-5].

In practice, chickens are given antibiotics for either treatment or prophylaxis of infections, and for growth promotion to increase profits. These antibiotics belong to similar chemical categories to those used for treatment of microbial human infections. This raises concern on the possibility of human cross-infecting with chicken-infecting bacteria or the later transferring resistance traits to bacterial population that causes human infections [6-9].

Previous studies have identified different types of bacteria in chicken meat and shown high antimicrobial resistance rates [10-15]. The present study intended to isolate bacteria from chicken fecal materials and assess antimicrobial resistance profiles with an ultimate goal of raising awareness among chicken keepers and policy makers on judicious use of antibiotics to prevent further spread of antibiotic resistance.

### MATERIALS AND METHODS

#### Sample collection, Isolation and Identification of microorganisms

Samples of fresh chicken droppings were collected from chicken keepers residing in 13 localities situated within 3 municipalities namely Ilala, Temeke and Kinondoni. The localities were situated within a radius of 30 kilometers from Dar es Salaam City center [fig. 1]. The samples were collected by using sterile spatula and deposited into closed sterile bottles prior transporting them to our laboratory for further processing, on the same day. Five grams of each sample was suspended in about 5 ml of sterile normal saline and left for 5 minutes to sediment. Fifty microliters of supernatant was drawn and spread-plated onto freshly prepared Nutrient agar (Carl Roth, Germany) and incubated overnight at 37°C. Identification of the isolated bacteria was performed using selective and differential media and confirmed by biochemical tests as previously described [16].



Key: Stars depict the localities where chickens' droppings were collected

Fig. 1: The studied areas/localities of Dar es Salaam region

#### Antibiotic susceptibility and multiple antibiotic resistance (MAR) determination

Each identified bacterial isolate was subjected to antibiotic resistance profiling against 9 widely used antibiotic viz. Gentamicin (10µg), Ceftriaxone (30µg), Amikacin (30µg), and Chloramphenicol (30µg)-(Oxoid, United Kingdom); and Ciprofloxacin (5µg), Oxacillin (1µg), Co-trimoxazole (25µg) and Erythromycin (15µg) as well as Ampicillin (30µg)-(Bioanalyse, Turkey). All assays were performed in Mueller-Hinton agar plates (Carl Roth, Germany) using the Kirby-Bauer disk-diffusion method. Each identified bacterial isolate was re-suspended into Ringer's lactate solution for 2-4 h and compared to 0.5 McFarland standard turbidity (equivalent to  $1.5 \times 10^8$  colony forming unit per millilitre (cfu/ml); prior to subjecting them to antibiotic susceptibility analysis as per Clinical Laboratory and Standards Institute (CLSI) guidelines [17]. Each of the above procedures was done in triplicate for statistical purpose and consistency of results; therefore the numerical values are expressed as means. Four strains of reference bacteria from the American Type Culture Collection (ATCC) namely *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC700603), *Pseudomonas aeruginosa*

(ATCC27853) and *Staphylococcus aureus* (ATCC 25923) were employed as control bacteria.

Multiple antibiotic resistance (MAR) index was determined using the formula  $MAR=y/x$ , where  $y$  = was total number of antibiotics to which test isolate displayed resistance; and  $x$  = total number of antibiotics to which the test bacteria were evaluated for sensitivity [18].

## RESULTS

### Isolated bacteria from the study areas/localities

Thirteen localities from the 3 municipalities of Dare s Salaam were surveyed [fig. 1]. From those a total of 100 samples of chicken droppings were collected, and 188 bacteria were isolated and identified. The identified bacteria were comprised of five genera namely *E. coli*, *K. pneumoniae*, *S. typhi*, *P. aeruginosa* and *S. aureus*. Almost 50% of the bacteria were *E. coli* and 6% was *P. aeruginosa* [table 1].

### Susceptibility profiles of the isolated bacteria

The sensitivity patterns of each species of the bacteria to antibiotics frequently used for treatment of human ailment were compared to that of respective control strains of bacteria as described below:

#### *Escherichia coli*

Significant differences in IZ were observed between isolates of *E. coli* and the control bacterium/*E. coli* (ATCC25922) when these were tested against Chloramphenicol, Ciprofloxacin and Ceftriaxone ( $p = 0.001$ ) as shown in [fig. 2]. The highest resistance rate was exhibited against Co-trimoxazole (65.8%) while more than half (69.3%) of the *E. coli* isolates were sensitive to Gentamicin (table 1).

#### *Klebsiella pneumoniae*

Statistical comparison of IZ between isolated bacteria and the control bacterium (*Klebsiella*), revealed significant differences for Chloramphenicol ( $p=0.04$ ) and Co-trimoxazole ( $p=0.03$ ).

High antibacterial resistance rate was exerted against Co-trimoxazole (64%) as compared to 13.3% exerted against Gentamicin. Most isolates (73.3%) were sensitive to Ampicillin and Ceftriaxone (80%) as shown in [fig. 2] and [table 1].

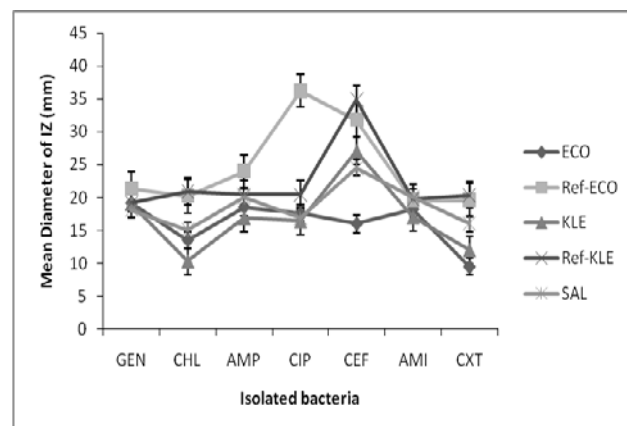


Fig. 2: Comparison of susceptibility patterns of isolates of *E. coli*, *S. typhi* and *K. pneumoniae* to their respective control bacteria.

Table 1: Resistance profiles of the isolated bacteria against the commonly used antibiotics

Antibiotics	Bacteria/Susceptibility Profiles (%)				
	ECO (n=82)	KLE(n=22)	SAL(n=22)	PSE(n=18)	STA(n=44)
GEN	S(69.3); I(19.7); R(11.0)	S(73.3); I(13.3); R(13.3)	S(53.3); I(26.7); R(20.0)	S(55.6); I(22.0); R(22.4)	S(80.6); I(19.4); R(0.0)
CHL	S(18.0); I(42.0); R(39.0)	S(3.4); I(19.3); R(77.3)	S(26.7); I(40.0); R(33.3)	S(11.0); I(33.0); R(56.0)	S(49.0); I(30.6); R(20.4)
AMP	S(50.0); I(30.5); R(19.5)	S(46.4); I(40.0); R(13.6)	S(60.0); I(30.9); R(9.1)	S(63.5); I(36.5); R(0.0)	S(56.4); I(26.7); R(15.9)
CIP	S(33.3); I(38.7); R(28.0)	S(40.0); I(14.6); R(45.4)	S(41.0); I(27); R(32.0)	S(6.4); I(32.6); R(61.0)	S(10.4); I(44.1); R(54.5)
CEF	S(33.4); I(6.6); R(50.0)	S(80.0); I(6.4); R(13.6)	S(76.4); I(17.6); R(0.0)	S(15.4); I(29.1); R(55.5)	S(2.4); I(45.3); R(52.3)
AMI	S(46.3); I(53.7); R(0.0)	Nd	S(68.3); I(18.1); R(13.6)	S(55.8); I(22.0); R(22.2)	S(1.4); I(62.8); R(31.8)
ERY	Nd	Nd	Nd	Nd	S(30.6); I(19.4); R(50.0)
OXA	Nd	Nd	Nd	Nd	S(61.0); I(11.0); R(28.0)
CXT	S(14.2); I(19.4); R(65.8)	S(14.0); I(9.0); R(64.0)	S(28.5); I(14.0); R(59.1)	Nd	Nd

Keys: ECO-*E. coli*; KLE-*K. pneumoniae*; SAL-*S. typhi*; PSE-*P. aeruginosa*; STA-*S. aureus*; GEN-Gentamicin; CHL-Chloramphenicol; AMP-Ampicillin; CIP-Ciprofloxacin; CEF-Ceftriaxone; AMI-Amikacin; ERY-Erythromycin; OXA-Oxacillin; CXT-Co-trimoxazole. S-susceptible; I-intermediate; R-resistant; Nd-not done

#### *Salmonella typhi*

Similarly the susceptibility patterns of the isolated *Salmonella typhi* differed significantly from that of control *E. coli* (ATCC25922) when these were tested on Chloramphenicol and Co-trimoxazole with  $p=0.01$  and  $p= 0.003$  respectively. The highest resistance rate (59.1%) was exerted against Co-trimoxazole while none exhibited resistance to Ceftriaxone [table 1].

#### *Pseudomonas aeruginosa*

Significant differences in IZ were exhibited between the isolates of *P. aeruginosa* in comparison to control *P. aeruginosa* (ATCC 27853) when these were assayed against Chloramphenicol ( $p=0.001$ ),

Ceftriaxone and Ciprofloxacin both with  $p$ -values of 0.01. The highest antibiotic resistance rates were exerted against Ciprofloxacin (61%) and Chloramphenicol (56%) as depicted in [table 1] and [fig. 3].

#### *Staphylococcus aureus*

The highest antibacterial resistance rate exhibited by the isolated *S. aureus* was 54.5% against Ciprofloxacin, and none was resistant to Gentamicin as shown in [table 1].

When the IZ produced by isolates of *S. aureus* were compared to the control bacterium, significant differences were revealed against Ceftriaxone ( $p =0.03$ ), Ciprofloxacin ( $p =0.01$ ),

Chloramphenicol ( $p=0.04$ ), as well as against Amikacin and Erythromycin ( $p=0.02$ ) as shown in [fig. 3].

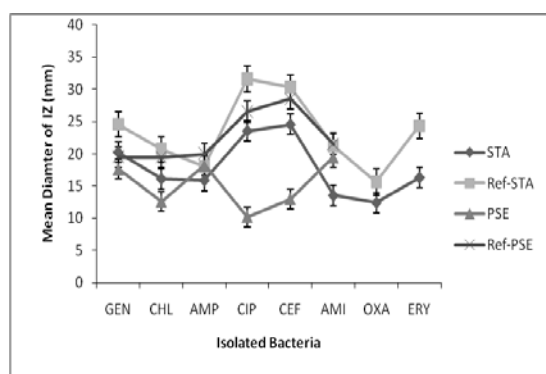


Fig. 3: Comparison of susceptibility patterns of isolates of *S. aureus* and *P. aeruginosa* to their respective control bacteria.

### Multiple antibiotic resistance indexing

The multiple antibiotic resistances (MAR) index was determined as the ratio of number of antibiotics to which the bacterium was resistant to a total number of antibiotics to which bacterium was exposed [19]. MAR index values greater than 0.2 indicated high risk source of antibiotic exposure or contamination where antibiotics are often used [20]. Calculated MAR [table 2] suggests that almost all the test bacteria exhibited multiple antibiotic resistance rates in the following order: *E. coli*>*P. aeruginosa*>*K. pneumoniae*>*S. aureus*.

### DISCUSSION

Currently, antimicrobial resistance is a major health concern worldwide.

A number of factors attributable to antimicrobial resistance have been reported though measures to counteract them are being very slowly implemented. Irrational prescription and use of antimicrobial agents for human health problems, use of antimicrobial agents in agriculture and veterinary medicine have been highlighted as some of the main causes of the problem [21-22].

The use of antibiotics in poultry birds husbandry for growth fastening process, instead of treating or preventing bacterial infections, is apparently because of adjustment of intestinal flora favoring "good" bacteria while suppressing "bad ones" that provoke inflammation of the gut mucosa [23]. This practice was acknowledged by majority of chicken keepers interviewed in our study. For that matter, feeding chicken with antibiotic-mixed feed stuff may play an important role in an emergence of antimicrobial resistance in both human and poultry bird populations and/or other domestic animals as well as wild animals that live in proximity to human settlements. The bacteria isolated from chicken droppings exhibited multiple antibiotic resistances that is a clear indication of irrational and irresponsible use of antimicrobial agents, which might have an important role in the development of antimicrobial resistance, in both human and animals. The most effective antibiotics against the isolated bacteria were the Aminoglycosides (Gentamicin and Amikacin) followed by Ciprofloxacin; whereas Chloramphenicol was the least effective. And, this could be due to the fact that Aminoglycosides, particularly Gentamicin is relatively more expensive and thus not frequently used in treatment of neither human nor veterinary bacterial-associated illnesses.

The MAR indices determined in this study [table 3] is a good indication that a very large proportion of the isolated bacteria had been exposed to several antibiotics. Multiple antibiotic resistance exhibited by *E. coli*, *S. aureus*, and *P. aeruginosa* isolates is of major health concern since these are the main causes of health care facility acquired bacterial infections, particularly in immunocompromised individuals [24, 25].

Table 2: Multiple antibiotic resistance (MAR) indices of isolated bacteria from chicken droppings

MAR index	Frequency of MAR index (%)				
	ECO (n=82)	KLE (n=22)	SAL (n=22)	PSE (n=18)	STA (n=44)
0	7(8.5)	0(0.0)	0(0.0)	3(16.7)	1(2.3)
0.1	16(19.5)	8(36.4)	9(41.0)	1(5.6)	18(40.9)
0.2	8(9.8)	4(18.2)	1(4.5)	1(5.6)	2(4.6)
0.4	19(23.2)	2(9.1)	3(13.6)	4(22.2)	9(20.5)
0.5	7(8.5)	5(22.7)	0(0.0)	0(0.0)	5(11.4)
0.6	4(4.8)	0(0.0)	3(13.6)	7(38.9)	0(0.0)
0.7	13(15.9)	1(4.5)	1(4.5)	2(11.0)	3(6.7)
0.8	8(9.8)	0(0.0)	0(0.0)	0(0.0)	6(13.6)
1.0	0(0.0)	2(9.1)	5(22.8)	0(0.0)	0(0.0)

None of the tested antibiotics was effective against all isolated bacteria even those with broad antimicrobial spectra. This again, should be taken seriously because of poor availability and affordability of antimicrobial agents to combat the overwhelming microbial infections and other infectious tropical diseases in the country. Moreover, most Tanzanians cannot afford to buy new generation of antibiotics that are usually more effective but also expensive. Consequently, in most cases they are obliged to empirically use ineffective antibiotics, which are available and most importantly affordable. This has a serious health impacts on patients as could spell to exacerbate development of antibiotic resistance that may ultimately lead to death.

Five genera of bacteria were isolated from chicken droppings in the present study namely *E. coli*, *K. pneumoniae*, *S. typhi*, *P. aeruginosa* and *S. aureus*; though their bioburden differed significantly of which was not a scope of this study; most of the bacteria are human opportunistic pathogens. Of the isolated bacteria, *E. coli* constituted the largest fraction in comparison to other enteric bacteria as it is a natural inhabitant of the intestinal tracts of humans and warm-blooded animals; and thus it is used as an indicator bacterium for enteric zoonotic agent and for its faster acquisition of antimicrobial resistance than other conventional bacteria [26]. *Pseudomonas*

*aeruginosa* and *S. aureus* are some of problematic health facility associated pathogens that often express multidrug resistance [27].

Most of the known bacterial infections caused by *E. coli*, *Pseudomonas* and *Staphylococcus* species are contracted from undercooked meat or from drinking contaminated water, or from surface contamination of raw produce such as vegetables. Given that chicken droppings are applied on farm crops as manure, the antibiotic-resistant bacteria that persist in chicken manure can thus be transmitted to human through consumption of vegetables and under cooked meat stuffs. Large piles of aging chicken manure that are used as fertilizer on farm crops may fail to keep the microorganisms from reaching people through contaminated food or drinking water; however chicken manure is not treated before it is applied to farm fields. Consequently, the emergence of antibacterial resistance in food animal could be associated to the consumption of antimicrobials in veterinary medicine. Moreover, previous studies indicate that antibiotic resistant *S. typhi* could also be transmitted without selective pressure and more intricate mechanisms may be involved in the emergence of resistance among such bacteria [28, 29].

The observed variation of type of enteric bacteria in chickens is largely influenced by the type of feeds and source water that the chickens ingest, and the amount and frequency of the antimicrobial agents that they have been treated with or fed along with food [30]. But also the variation observed in the antibiotic resistance profiles of the bacteria in general, could be the consequence of indiscriminate use of antibiotics that could have led to the development of resistance as antibiotic resistance genes transfer to other pathogenic bacteria present in the gastrointestinal tract. This process may have undesirable clinical implications within human and livestock population having contact with such resistant pathogens. In view of that, this study has revealed the presence of bacterial isolates resistant to Ampicillin, Chloramphenicol and Co-trimoxazole, which are the most commonly, used antibiotics in Tanzania. This becomes a threat to our population as these are the most affordable and thus widely used antibiotics. Although Gentamicin is another commonly used antibiotic, but resistance rate against the antibiotic is comparatively low along with Ciprofloxacin and Ceftriaxone; and one of the factors is their relative higher price that affect their easy availability, which prevents them from creating selective pressure among the bacteria [31]. The bacteria from chicken droppings have exhibited antibiotic resistance, of which some of them were resistant to more than two antibiotics. The Gram negative bacteria showed high prevalence rate of antibacterial resistance to Chloramphenicol and Ciprofloxacin that are drugs of choice for treatment of Salmonella-associated infections in Tanzania. In conclusion, results indicated that unless deliberate measures are immediately adopted, chicken rearing could become a potential source of antibacterial resistant microorganisms that are transmitted via the food chain. Therefore, indiscriminate use of antibiotics should be controlled to prevent them from becoming obsolete. We also call for further studies that will involve larger study areas with more representative samples size to determine the magnitude of this problem nationwide and design appropriate means to prevent further development and spread of antibiotic resistance.

#### CONFLICT OF INTERESTS

Declared None

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