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 where 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 to 0.5 McFarland standard turbidity (equivalent to 1.5×10^8 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 pneumonia (ATCC700603), Pseudomonas aeruginosa (ATCC27853), and Staphylococcus aureus (ATCC29213) were used as controls. The bacterial species were identified using biochemical tests described as previously described [16].

Assessment of antibiotic resistance profiles

Each identified bacterial isolate was subjected to antibiotic resistant testing 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 Amoxicillin (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 1.5×10^8 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 pneumonia (ATCC700603), Pseudomonas aeruginosa (ATCC27853), and Staphylococcus aureus (ATCC29213) were used as controls. The bacterial species were identified using biochemical tests described as previously described [16].

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Antibiotic susceptibility and multiple antibiotic resistance (MAR) determination

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

Key: Stars depict the localities where chickens’ droppings were collected
Antibiotics

<table>
<thead>
<tr>
<th>Bacteria/Susceptibility Profiles (%)</th>
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<tbody>
<tr>
<td>ECO (n=82)</td>
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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 Ce-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].

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].

**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.

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 selection 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 Ce-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 Ce-trimoxazole; 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, 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 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.

Conflict of interests
Declared None

References
13. Mthembu MS. The usefulness of multiple antibiotic resistance indexing techniques in differentiating faecal coli bacteria from different sources. Thesis (MSc) University of Zululand; 2008.