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Antimicrobial resistance profile of bacterial isolates from fecal and cloacalswab samples collected from broiler chickens in Chennai, Southern India

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ABSTRACT

This study was aimed to analyze the antimicrobial resistance profile of bacterial isolates from fecal and cloacalswab samples collected from healthy broiler chickens in Chennai. Fresh fecal droppings were collected in sterile containers and cloacal swabs were collected using nylon swabs and brought to the laboratory with cold chains. Bacterial strains were isolated from the collected samples using standard bacterial culture methods. Antimicrobial susceptibility of the isolates was screened using Kirby-Bauer disk diffusion method. Extended spectrum β-lactamase (ESBL), metallo-β-lactamase (MBL), and AmpC β-lactamaseproducers were identified using Combination Disk Method. A total of 58 bacterial strains were isolated from fresh fecal droppings and 84 from cloacal swabs collected from broiler chickens. *Escherichia coli* (117*)* was the predominantly isolated bacteria followed by*Klebsiellapneumoniae*(17). Bacterial isolates showed 100% resistance to ampicillin, followed by piperacillin (97.8%), polymixin-B (87.3%), piperacillin-tazobactam (85.2%), tetracycline (77.4%), andnalidixic acid (65.4%). These isolates showed highest susceptibility to fosfomycin (96.4%). ESBL production was found among 59.1% of the isolates and AmpC β-lactamase production was noted among 43.6% of the isolates. None of the bacterial isolates were tested positive for MBL production. This study finding revealed that fecal waste of broiler chickens contains β-lactamase producing multidrug-resistant bacterial strains.

Key words: Broiler chicken, antimicrobial resistance, extended spectrum β-lactamase, metallo-β-lactamases, Escherichia coli.

1. Introduction

Poultry is one of the most widespread food industries and broiler chicken is the most commonly farmed species worldwide with over 90 billion tons of chicken meat produced per year (1). Antibiotics have been used in poultry for the prevention of disease, such as feed proficiency enhancers and growth promoters (2). Multiple combinations of antibiotics were used for the animal feeding process,releasing large quantities of antibiotics and entertain the cycle of biotransformation andbioaccumulation of antibiotics in the environment (3).Animal farms account for over one-half of all antibiotics used and it was estimated that 131,109 tons of antibiotics were used in 2013 and it will bereaching over 200,000 tons by 2030 (4).Furthermore, it is estimated that antibiotic utilization will be increased as 67% by the year 2030 and with almost twice this increase in countries such as China, Brazil, India, South Africa, and Russia (5, 6). Indiscriminate usage of essential human medicine antimicrobials in animal production is accelerating the emergence of multidrug-resistant (MDR) bacterial

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pathogens (7, 8). Discharge of antibiotic resistant genes from poultry to soil and aquatic environments can propagate by horizontal gene transfer (HGT) to indigenous bacteria (9).A study reported that poultry is a source of ESBL producing bacterial isolates which can be spread into the natural environment as well as our food chain (10). Bacterial isolates from healthy broilerchickens have significant resistance prevalence toβ-lactam antibiotics,especially tothe third and fourth generationcephalosporins,andthese bacteria can spread to humans and other animals (11, 12).Among poultry bacterial pathogens belonging to Enterobacteriaceae, avian pathogenic *Escherichia coli* (APEC) isolates displayed a high level of resistance to ampicillin, amoxicillin, and tetracycline (13).In this study, bacterial strains isolated from fecal and cloacal samples collected from broiler chickens in Chennai were screened for their antimicrobial resistance (AMR) profile.

2. Materials and Methods

Sample Collection and Bacterial Isolation

Fecaland cloacalsamples were collected from commercially available broiler chicken shops in Chennai. Pre-sterilized air-tightened containers were used for collection of fecal samples and a nylon swab was used for collection of cloacal swab samples. After immediate collection, samples were brought to the laboratory in the cold chain. For the isolation of bacterial strains, a loop full of fecal samples and cloacalswabs were inoculated on MacConkey Agar and 5% sheep blood agar platesand incubated at 37ºC for 18-24h. Isolated colonies were identified based on the standard bacterial culture and biochemical tests (14).

Antibiotic Susceptibility Test

Antibiotic susceptibility profile of the bacteria was screened using Kirby-Bauer disc diffusion method (15) using the antibiotics viz. amikacin (30mcg), ampicillin (30mcg), chloramphenicol (30mcg), ciprofloxacin (5mcg), ertapenem (30mcg), fosfomycin (200mcg), gentamicin (10mcg), meropenem(10mcg), nalidixic acid (30mcg), nitrofurantoin (300mcg), piperacillin (100mcg), piperacillin-tazobactam (100/10mcg), polymixin-B(300 units), and tetracycline (30mcg) (Himedia, India).

Combination Disk method for ESBL, MBL and AmpC

The cefotaxime (30µg) and cefotaxime – clavulanic acid (30µg/ 10 µg) discs were placed 20mm apart on the MHA agar surface. Similarly, ceftazidime (30µg), ceftazidime – clavulanic acid (30µg/ 10 µg) were also placed for detection of ESBL production using Combination Disk Method (CDM). In this method, an overnight culture suspension of the bacterial isolate was adjusted to 0.5 McFarland's standard and lawn culture was made on the surface of Muller Hinton Agar (MHA) plates. Imipenem(10µg) alone and in combination with EDTA (750µg) were used for detection of MBL production. Cefoxitin (30 µg) and cefoxitin-cloxacillin (30 µg/ 200 µg) discs were used for AmpC production.After overnight incubation at 37^0C, an increased zone of inhibition more than 5mm was interpreted as positive for β-lactamaseproduction.

3. Results

In this study, a total of 46 fresh fecal droppings and 60 cloacal swabs were collected from broiler chickens in Chennai. Among the collected samples, 142 gram-negative bacterial strains were isolated. Out of them, 58 (40.8%) bacterial isolates were obtained from fecal samples and 84 (59.1%) were obtained fromcloacal swabs.Among the 142 isolates, 117 (82.4%)were *E.coli*, 17 (11.9%) *K.pneumoniae*,3 (2.1%) *Proteus vulgaris*, 3 (2.1%) *P.mirabilis*, 1 (0.7%) *Enterobacter sp.*and1(0.7%)*Morganella sp.* (Fig.1).

All isolates were screened for antibiotic resistance profile using standard antibiotics. It was noted that bacterial isolates showed high level of resistance to ampicillin (100%),followed by piperacillin/tazobactam (97.9%),piperacillin (93.9%),polymixinB (81.6%),tetracycline (79.6%),meropenem (65.3%), gentamicin (55.1%),ertapenem (53.1%) and nalidixic acid (53.1%). The isolates were found to be highly susceptible to fosfomycin (97.9%) followed by chloramphenicol (69.4%), amikacin (67.3%), nitrofurantoin (59.2%) and ciprofloxacin (57.1%) (Fig.2).

E.coli showed high level of resistance to ampicillin(97.6%) followed by piperacillin (92.8%), piperacillin/tazobactam (95.2%), tetracycline (80.9%), polymixin B (78.5%),meropenem (69%),gentamicin (54.7%) and ertapenem(52.3%) and high level of susceptibility to fosfomycin(97.6%) followed by amikacin (69%),nitrofurantoin (66.6%) and ciprofloxacin(57.1%). *K.pneumoniae*showed 100% resistance to ampicillin, piperacillin,piperacillin/tazobactam, and polymixinB followed by tetracycline (80%),ertapenem(60%) and 100% susceptibility to fosfomycin. *P.vulgaris*showed 100% resistance to ampicillin, piperacillin/tazobactam, polymixin B, and piperacillin and 100% sensitivity toamikacin, fosfomycin and tetracycline.*P.mirabilis* showed 100% resistance to ampicillin,ciprofloxacin, ertapenem, gentamicin,meropenem,piperacillin/tazobactam, polymixin B, and piperacillinand 100% sensitivity to amikacin,chloramphenicol,fosfomycin, nalidixic acid and nitrofurantoin (Fig. 2).Phenotypic detection of β-lactamases revealed that 49 isolates showed positive for ESBL production. Of them,42 (85.7%) were*E.coli*,5(10.2%) were *K.pneumonia*,andeach1 (2%) was*P.vulgaris* and*P. mirabilis*. All β–lactamase producing isolates possessed MDR profile against at least 4 antibiotics used in this study.Out of 49 isolates, 46(97.9%) showed positivity forAmpC productionand among them 41(97.6%) were *E.coli*, and5(10.2%) were*K. pneumoniae*(Fig.3).

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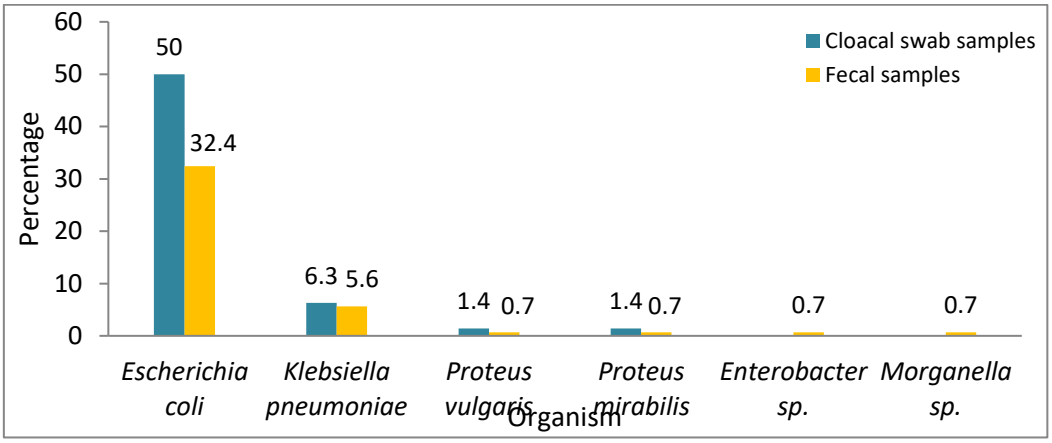

Fig.1: Percentage of bacterial species isolated from fecal and cloacal samples from broiler chicken

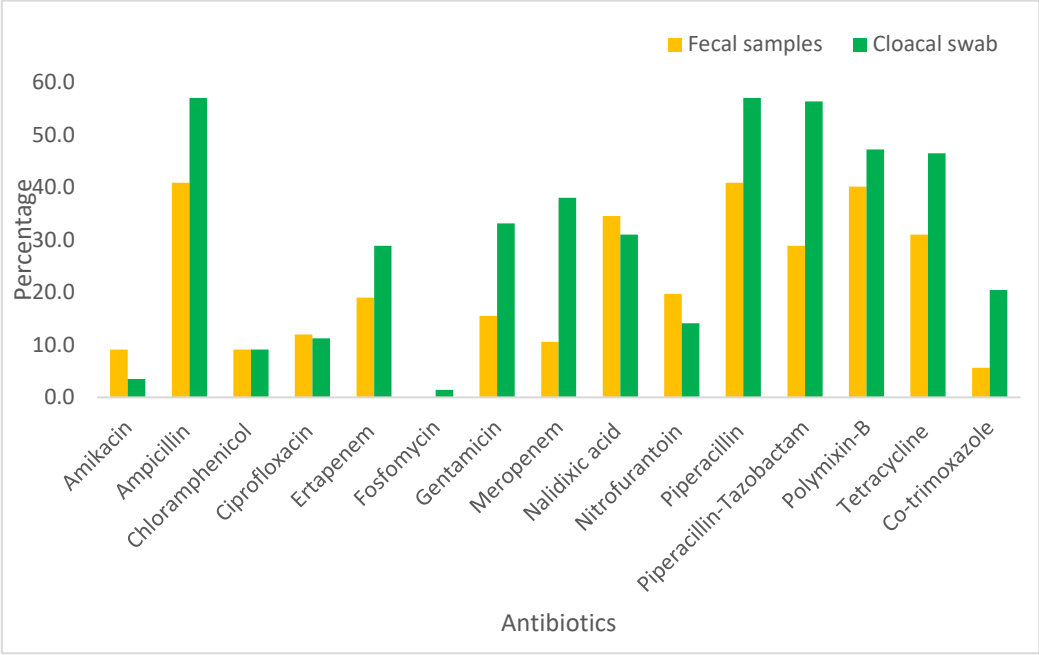

Fig. 2: Antibiotic resistance profile of bacterial isolates from fecal and cloacal swab samples collected from broiler chickens in Chennai

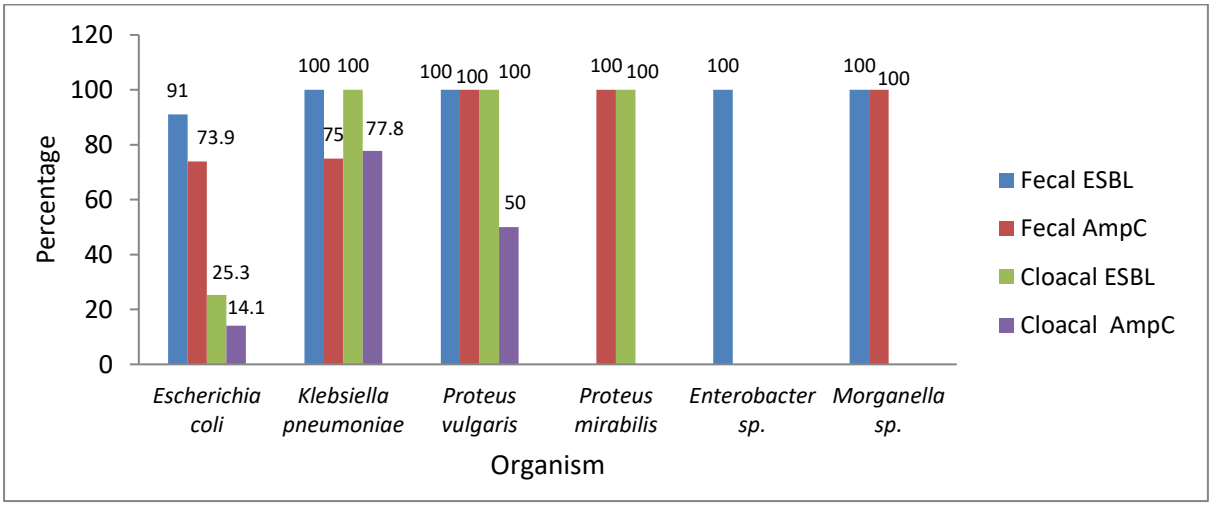

Fig. 3: Positivity of ESBL and AmpCproduction among bacterial isolates from fecal and cloacal swab samples collected from broiler chickens

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4. DISCUSSION

Antimicrobial drugs are used for treating infectious diseases in animals and humans.In animal husbandry, particularly poultry farms, antimicrobials are widely used to increase productivity. However, the uncontrolleduse of antimicrobials in livestock and poultry is one of the major reasons forthe emergence and spread of antibiotic resistant bacteria. It is also posed arisk to public health through potential transfer of AMR genes to human pathogens, through contact with animals or through the food chain. AMR therefore presentsa threat to animal and human health and economy (16). In our study, the AMR pattern ofbacterial isolates from fecal and cloacal swab specimens collected from broiler chickens was analyzed. The most common bacterial species isolated in this study was*E.coli* followed by*K.pneumoniae.* Other bacterial species such as *P. vulgaris, P.mirabilis, Enterobacter sp.,* and *Morganella sp.* were isolated in low levels. In a study from Saudi Arabia,*E. coli* was the predominantly isolated bacteria from chicken farms. Other than *E.coli,Salmonellasaprophyticus, S. epidermidis, Proteus sp., Enterococcus sp., Klebsiella sp., Bacillus sp., Pseudomonas sp.* and *Micrococcus sp.*were also identified in their study in samples collected from chickens(17).However, in this current study, none of the *Salmonella sp.*was isolated from both fecal and cloacal swab specimens. A study by Roy *et al.*(18) reported that *E.coli*was the most prevalent bacterial isolate frompoultry chickens.

In this current study, while analyzing the overall antibiotic resistant profile of Gram-negative bacterial pathogens, it was noted that bacterial isolates showed high levels of resistance to beta-lactam antibiotics such as penicillin and piperacillin. More than 50% of resistance rate was found topolymixin B, tetracycline, meropenem, gentamicin, ertapenem, and nalidixic acid. This AMR profile reveals that beta-lactam antibiotics, carbapenems, and aminoglycosides and tetracycline antibiotics are highly used in poultry farms. In poultry farms located in Northern America, tetracycline, bacitracin, tylosin, salinomycin, virginiamycin,and bambermycin are often used. Around 30% to 90% of the antibiotic dose consumed is released in the urine and feces. Hence, sewage disposal system is one of the main routes for antibiotics entering into the environment and contaminatedthe water system (19). A study by Diarra*et al.* (19) reported that bacterial isolates from poultry farms showed a resistance rate of more than 43% to ampicillin, amoxicillin-clavulanic acid, ceftiofur, cefoxitin and ceftriaxone antibiotics.

Amsaveni*et al.*(20) reported the high positivity of ESBL producing*E.coli* isolates from poultry farms. In this study, high positivity of ESBL production was noted among *E.coli*isolates. It was also noted that *K. pneumoniae, P. vulgaris,* and *P. mirabilis* exhibited low levels of ESBL positivity. Since none of our study isolates showed positive for MBL production, other carbapenemases could be responsible for the ertapenem resistance observed in this study isolates. Positivity of MBL producing bacteria isolated from healthy broiler chickens might be the major concern in health,so the transmission might occur between poultry bacteria with human pathogens (21).Another study reported a low level of MBL production among bacterial strains isolated from poultry farms(7). Furthermore, in this study,AmpC positivity was observed among 25.3% of the isolates. The AmpC positivity in

this study is higher than a study by Chika *et al.*in 2016 (22) reported 18.4% of the AmpCproduction of bacterial strains from poultry. From the findings of this study, it is concluded that the bacteria isolated from fecal and cloacal swab specimens collected from health broiler chickens in Chennai have multidrug-resistance profile. Furthermore, the high positivity ESBL production could be the major factor for bacterial resistance to cephalosporin antibiotics.

Conflict of interest: None to declare.

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