ORIGINAL PAPER

Efficacy of quinolones and cephalosporins against antibiogram of *Escherichia coli* isolated from chickens

Ranjhan Ali Lakho[1], Shahid Hussain Abro[1], Muhammad Tarique Tunio[2*], Mohsina Zubair[3], Rani Abro[4], Rahmatullah Rind[1], Riaz Ahmed Leghari[1], Kanwar Kumar Malhi[1], Muhammad Rafique Rind[5], and Asghar Ali Kamboh[1]

Key Message This study evaluates the efficacy of quinolones and cephalosporins against antibiogram of *E. coli* isolated from chicken flocks and it reveals that these antibiotics were found to be effective against *E. coli*.

ABSTRACT Colibacillosis is an acute septicemia disease caused by *E. coli* producing considerable morbidity and mortality especially in poultry. Quinolones and cephalosporins have been used in treatment of various infections. Therefore, this study was designed to evaluate the efficacy of quinolones and cephalosporins against the antibiogram of *E. coli* isolated from chicken flocks. The 100 blood samples from liver and intestine were collected from different poultry vendors surrounding of Tandojam and Hyderabad, Sindh, Pakistan. The isolated organism was cultured on nutrient and blood agar media. The cultural, morphological and biochemical characteristics were observed for the confirmation of the isolated organism. The minimum inhibitory concentration (MIC) of different antibiotics against *E. coli* was performed by serially diluting antibiotics ciprofloxacin, metronidazole, cefipime as 0.4 µg/ml, 0.8 µg/ml, 1.6 µg/ml, 3.2 µg/ml and 6.4 µg/ml, 12.5 µg/ml, 25 µg/ml and 50 µg/ml, respectively. The mean zones of inhibition of ciprofloxacin, ofloxacin, enrofloxacin, norfloxacin, cefepime, ceftazidimine and cefoxitin against the antibiogram of *E. coli* were recorded as 14, 11, 12, 11, 11, 11, and 11 mm, respectively. MIC results indicated that ciprofloxacin was found to be more effective to inhibit the growth of *E. coli*. It was found that antibiotics of quinolones group namely ciprofloxacin, enroflaxcin and ofloxacin as well as cephalosporin group namely cefipime, ceftazidime and cefoxitin were found to be effective to isolate *E. coli*.

Key words Antibiogram, Cephalosporins, Chicken, *E. coli*, Quinolones

[1]Department of Veterinary Microbiology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam 70060, Pakistan
[2]Department of Agricultural Sciences, Faculty of Sciences, Allama Iqbal Open University, Islamabad
[3]Department of Chemistry, Preston University, Islamabad
[4]Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam 70060, Pakistan
[5]Department of Biotechnology, Sindh Agriculture University, Tandojam 70060, Pakistan

*Corresponding author: Muhammad Tarique Tunio (tarique_tunio@hotmail.com)

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INTRODUCTION

Pakistan is basically an agricultural country and livestock is considered to be an important sub-sector which contributes about 52.2% to the value addition in the agriculture sector and 11% in the total GDP (Khan et al., 2008). In Pakistan, poultry industry is developing during the last couple of decades and contributing in the increase of GDP. This sector not only produces meat and eggs but also provides large scale employment opportunities and source of income for the people (Government of Pakistan [GOP], 2014); Anjum et al., 2016). Microbial agents especially bacterial pathogens produce considerable health and production losses in poultry.

In Pakistan, colibacillosis is one of devastating diseases that causes heavy economic losses in poultry industry. This disease produces acute septicemia disease caused by *E. coli* with heavy motility particularly in broilers (Shah et al., 2004). *E. coli* were discovered by German Pediatrician and bacteriologist Theodor Escherichia in 1885. *E. coli* is widely distributed in nature and commonly found in normal intestinal tracts of man, animals and birds. It is a gram negative and rod shape bacterium, there are approximately 100 serotypes that have been recognized but *E. coli* strains 01, 02 and 078 are the most pathogenic causing severe infections in poultry and survive for longer time in poultry house. Usually, the organisms are present in the soil, water, dust, air, feed, litter, feathers and in open surfaces of the poultry farm (Holt et al., 1994). *E. coli* are not only harmful for poultry but also hazardous for other animal species as well.

E. coli can cause gastroenteritis, urinary tract infection, calf dysentery or "white scores" neonatal meningitis, mastitis, cystitis, metritis in cattle, sleeping foal disease in horse. It can cause peritonitis, pneumonia and in some cases it causes septicemia resulting animal death within 48 hours. It also causes enterotoxaemia in sheep and goats (Panduranga, 1996). In poultry, *E. coli* infected birds clinically show dullness, depression, elevated temperature, inappetence, diarrhea, hemorrhages and lesions on heart, intestines, proventiculous and gizzard (Cardona & Msoffe, 2009). There is growing concern to transmission and association of infections in humans. Recently, cephalosporin-resistant *E. coli* strains isolated from poultry exhibited phylogenetic relationship with the organism from human workers (Angela et al., 2016). The intestinal inhabitant commensally bacterial species poses a certain resistance against invasion and colonization of microbial agents (Pamer, 2016). The irrational use of antibiotics in veterinary practice is a great concern for treatment of bacterial infections. Also several microorganisms have acquired resistance to drugs, and decreases therapeutic options in clinical settings (Buffie et al., 2012).

Cephalosporins (β-lactum antibiotics; cefepime, cefotaxime, ceftriaxone and cefuroxime) are derived from fungus Acremonium. These antibiotics are commonly used in treatment of infection by gram negative bacteria i.e enterococci and *Streptococci pneumonia* (Palleres et al., 1993; Dahms et al., 1998). Cephalosporins induce their biological functions by interaction with bacterial enzymes and β-lactum (Flynn & Edwin, 2013). Quinolones (ciprofloxacin, ofloxacin, rifampicin, lincomycin, bacitracin, enrofoxacin, nalidixic acid and norfloxacin) are broad-spectrum antibiotics commonly used in veterinary medicine. It has been reported that these antibiotics lead to alteration, reduce accumulation and DN grease protection (Oliphant et al., 2002). In *E. coli*, quinolones target topoismerase IV for its antimicriobial action (Khodursky et al., 1995). Keeping in view the good therapeutic results of cephalosporins and quinolones against the gram negative bacteria, this study was designed to evaluate antimicrobial resistance/sensitivity of *E. coli* to quinolones and cephalosporins groups of antibiotics commercially available in market.

MATERIALS AND METHODS

Collection of samples, isolation and identification of the organism

The 100 blood samples of liver and intestine were collected from different poultry vendors in surrounding of Tandojam and Hyderabad, Sindh, Pakistan. The reference strain of *E. coli* was obtained from Department of Microbiology, University of Karachi. The samples were analyzed at Department of Veterinary Microbiology, Sindh Agriculture University Tandojam, Central Veterinary Diagnostic Laboratory, Tandojam, and Vaccine Production Unit, Tandojam, Hyderabad, Pakistan. The colonies of *E. coli* were used to obtain pure culture for the biochemical properties and sugar utilization efficiencies. The samples were cultured, isolated and identified using the methods described (Abro et al., 2016a, 2016b). The reference strain of *E. coli* was processed for confirming the similar cultural and biochemical characteristics.

Susceptibility test

The Muller Hinton agar (Difco) was prepared according to manufacturer's instructions and incubated at 37 °C for 15 min. Bulks of pure culture colonies were suspended in normal saline solution in order to match barium chloride standard for antibiotic sensitivity. Sterile swab was soaked in the suspended solution and culture was smeared on the medium. The colonies suspended swab was evenly applied on the surface of the medium and plates were incubated at 37 °C for 15 min. The antibiotics comprising of quinolones group; ciprofloxacin, ofloxacin, rifampicin, lincomycin, bacitracin, enrofoxacin, nalidixic acid, norfloxacin and Cephalosporins

group; cephradin, cefuroxime sodium, cefepime, cephalexin, ceftazidime and cefoxitin, cefotaxime, ceftriaxone and cefuroxime were used in this investigation. The antibiotic discs were applied on medium surface using disc dispenser and gently pressed with sterile forceps in order to complete contact with the surface of Muller Hinton agar. The plates were incubated overnight at 37 ^{o}C. The zone of inhibition was measured as a clear zone (free from growth around the disc = -) and a clear zone of inhibition formed against *E. coli*. The zone of inhibition produced by the drugs was measured from the center of disc to zone in millimeters.

Minimum inhibitory concentration (MIC)

This method is used to determine the minimum inhibitory concentration (MIC) of selected antibiotics. Muller Hilton broth was prepared in ten flasks, marked as 0.4 ug/ml. 0.8 µg/ml, 1.6 µg/ml, 3.2 µg/ml, 6.4 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml and control (c). The medium was then incubated overnight to check sterility. Selected drugs (ciprofloxacin, metronidazole and cefapime) were added in above mentioned nine flasks according to the dilution mark on them and tenth was kept as a control. Forty test tubes measuring 13 × 100 mm plugged with cotton were sterilized in hot air oven at 170 ^{0}C for 2 h. The test tubes were grouped into four. Test tubes were then filled with medium containing antibiotic at the rate of 5 ml per tube. A single colony from *E. coli* incubated in the Miller Hilton broth; following the next day the organism was inoculated into the test tubes of each antibiotics group comprising of quinolones and cephalosporins. The test tubes were then incubated over-night and growth was observed. The minimum inhibitory concentration (MIC) of different antibiotics against *E. coli* was performed by serially diluting antibiotics as 0.4 µg/ml, 0.8 µg/ml, 1.6 µg/ml, 3.2 µg/ml and 6.4 µg/ml, 12.5 µg/ml, 25 µg/ml and 50 µg/ml, respectively.

RESULTS AND DISCUSSION

The data regarding mean size of inhibition of various antibiotics against *E. coli* is presented in Table 1. Higher zones of inhibition were formed by ciprofloxacin, ofloxacin, enrofloxacin, norfloxacin, cefepime, ceftazidimine and cefoxitin and their mean zones of inhibition was 14.0, 11.0, 12.0, 11.0, 11.0, 11.0 and 11.0 mm, respectively. Previously, it has been reported that organism is highly sensitive to these antibiotics (Rozina et al., 2004). However, earlier research observed that increase in resistance of *E.coli* to different antibiotics; oxytetracycline (97-100%), tetracycline (95-100%), neomycin (62-71%), trimethoprim (95-98%) and amoxicillin (50-65%). The exposure of *E. coli* was detected in chicken flocks of various poultry farms in Pakistan. The sensitivity of bacitracin against the isolated *E. coli* was observed resistant therapeutic in the control of *E. coli* infection. The findings of Ogunbanwo and Onilude (2004) are contradicted with the findings of our study. It was revealed in this study that *E. coli* showed complete resistance against bacitracin. While other antibiotics showed smaller zone of inhibition and were recorded as moderate or less effective against *E. coli*. However, it was observed that *E. coli* showed complete resistance against the lincomycin and bacitracin (Table 1). The efficiencies of enrofloxacin (73.96%) and amikacin (67.71%) had been reported highly active against *E. coli* isolated from broiler chicken. Although enrofloxacin was found to be more superior in the antibiotic activity than that of oxytetracycline and sulfadiment for control of morbidity and mortality caused by *E. coli* (Saha et al., 2003). *In vitro* antimicrobial testing of the isolated *E. coli* had been reported that 14 antimicrobial drugs revealed that 90% of the isolates of *E. coli* were sensitive to gentamycin and gentadox. They recorded that the organism was moderate sensitive (30-65%) to amoxicillin, sulfamethoxazole/trimethoprim, doxystin, chloramphenicol, enrofloxacin, furozolidine, norfloxacin, neomycin and ciprofloxacin. The findings of current research demonstrated that quinolones group; ciprofloxacin, enroflaxacin and ofloxacin effective against the isolated organism. Whereas, cephalosporin group; cefipime, ceftazidime and cefoxitin showed good efficacy against the organism.

Table 1 The mean sensitivity of *E. coli* against the various antibiotics observed during study

S. No.	Antibiotic used	Code	Zone of inhibition (mm)
A	**Quinolones**		
1.	Ciprofloxacin	CIP5	14
2	Ofloxacin	OFX5	11
3	Rifampicin	RD5	02
4	Lincomycin	MY10	R
5	Bacitracin	B10	R
6	Enrofoxacin	ENR5	12
7	Nalidixic acid	NA30	07
8	Norfloxacin	NOR10	11
B	**Cephalosporins**		
9	Cephradin	CE30	06
10	Cefuroxime sodium	CXM30	07
11	Cefipime	FEP30	11
12	Cephalexin	CL30	5.6
13	Ceftazidime	CAZ30	11
14	Cefoxitin	FOX30	11

R- Resistant i.e. no zone of inhibition

Table 2 The minimum inhibitory concentration of different antibiotic against *E. coli*

	Concentration of antibiotics (µg per ml media)									
Antibiotic	0.4	0.8	1.6	3.2	6.4	12.5	25	50	100	Control
Ciprofloxacin	-	-	-	-	-	-	-	-	-	+
Metronidazole	+	+	+	+	+	+	+	+	+	+
Cefipime	+	+	-	-	-	-	-	-	-	+
Amikacin	+	+	+	-	-	-	-	-	-	+

+ = Growth, - = No growth

The minimum inhibitory concentration (MIC) of different antibiotics against *E. coli* was performed by serially diluting antibiotics such as ciprofloxacin, metronidazole and cefipime as 0.4-50 µg/ml (Table 2). MIC results indicated that ciprofloxacin was found to be effective to inhibit the growth of *E. coli*. While the MIC of cefepime was found to be elevated concentrations of 0.4 µg/ml and 0.8 µg/ml of *E. coli*. In this study, ciprofloxacin showed better efficacy than that of cefepime against the isolated *E. coli*. Shuyu Wu et al. (2008) investigated in their conducted research on E. coli and found plate showed 74% effective for all strains and agreed within ± 1 log2 dilution when comparing MICs with Mueller-Hinton II media. Whereas, they noted significant variations for oxytetracycline and sulfamethoxazole against the organism. The description regarding minimum inhibitory concentrations of *E. coli* are in accordance with the previous reports (Saha et al., 2003; Shareef, 2004; Shuvu et al., 2008). Our findings demonstrated that the organism was highly sensitive to ciprofloxacin.

CONCLUSION

The antibiotics of quinolones group; ciprofloxacin, enrofloxacin and ofloxacin were effective to isolate *E. coli*. Whereas drugs belonged to cephalosporin group; cefipime, ceftazidime and cefoxitin showed good efficacy against the organism. The similar MIC results were obtained for antibiotic of quinolones and cephalosporin. The antibiogram of *E. coli* indicated that ciprofloxacin found to be effective antibiotic of quinolones and cephalosporin groups.

Author Contribution Statement Ranjhan Ali Lakho and Shahid Hussain Abro contributed in study conception and design. Ranjhan Ali Lakho, Shahid Hussain Abro and Muhammad Tarique Tunio contributed in acquisition of data. Mohsina Zubair, Rani Abro, Rahmatullah Rind contributed in analysis and interpretation of data. Riaz Ahmed Leghari and Kanwar Kumar Malhi contributed in drafting the manuscript. Ranjhan Ali Lakho, Muhammad Rafique Rind, and Asghar Ali Kamboh contributed in critical revision.

Conflict of Interest There is no conflict of interest

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