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COMPARATIVE ANTIBIOTIC RESISTANCE PROFILE OF THE MULTIDRUG RESISTANT E.COLI ISOLATED FROM COMMERCIAL AND BACKYARD POULTRY

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ABSTRACT

Community acquired multidrug resistant *E. coli* has become an emerging issue worldwide. The present study was planned to compare the antibiotic resistance profile of *E. coli* isolated from commercial broilers and backyard poultry reared in close proximity to human population. Out of 200 cloacal swabs (100 each from commercial broiler & backyard poultry), 126 samples (n=70 commercial broiler, n=56 backyard poultry) were found positive for *E. coli* through *16S rRNA* PCR. The percentage of multidrug resistant *E. coli* isolates from commercial broiler chicken and backyard poultry were 64.2% and 53.5% respectively. Overall high antibiotic resistance was observed against Amoxycillin and Tetracycline with percentage resistance of 71.4% and 57.1% in commercial broiler, whereas 80.3% and 82.1% in backyard poultry respectively. The percentage resistance against Streptomycin and Sulphamethoxazole/Trimethoprim was observed to be high in backyard poultry (64.2% and 53.5%) as compared to the commercial broiler (28.5% and 35.7%) respectively. Percentage resistance against cephalosporin like ceftriaxone, cefaclor and ceftiofur was low both in case of backyard poultry and commercial broilers. Statistical analysis reveals that significant association exists between poultry species and antibiotic resistance of Oxytetracyclin, Streptomycin, Sulphamethoxazole/Trimethoprim, ceftriaxone, cefaclor and ceftiofur($p<0.05$). The present study revealed that the trend in antibiotic resistance percentage is increasing in the backyard poultry as compared to the commercial poultry, which is possibly due to their close community interaction.

Key words: Backyard poultry, *E. coli*, Multidrug resistant, *16S rRNA* PCR**.**

INTRODUCTION

Escherichia coli(*E. coli*), a gram negative versatile enteric microorganism belongs to Enterobacteriaceae family acting both as commensal and pathogenic organism. It is considered to be the major cause of morbidity as well as mortality both in animals and human worldwide (Miskinyte *et al.* 2013).*E. coli* of animal origin can acquire antibiotic resistance from multi drug resistant bacteria of soil and various food(Altalhi *et al.* 2010; Abdallah 2011) and this acquisition of gene transfer is suspected by the grazing of animal in that areas in which gene are transferred from soil to GIT of that animal (Srinivasan *et al.* 2008) as high level of antibiotic resistance genes have been observed in biosolids, manure and fertilized soil (Munir and Xagoraraki 2011). Gene transfer to animal are very common and are also occur by food chain (Koluman and Dikici 2013).It is a common practice of using the antibiotics in the commercial feed of animals and birds at sub therapeutic level for growth promotion.This phenomenon of antibiotic resistance is very common and it is assumed that the backyard poultry in Pakistan, which is for most of the time fed on waste and left over food of human and less on commercial feed, have less chances of antibiotic resistance as compared to commercial broiler flocks. On the other hand the commercial broiler flocks are fed on feed having added antibiotics in it for growth promotion and to camouflage the bad management. The present study has been designed to compare the antibiotic resistance pattern of *E.coli* isolated from commercial broiler chicken and backyard poultry.

MATERIALS AND METHODS

A total of (n=200)cloacal swab samples were collected from commercial broiler (n=100) and backyard poultry (n=100) suffering from collibacillosis.The samples were collected in a sterile container from collibacillosis suspected poultry birds which were brought to University Diagnostic Laboratory, University of Veterinary and Animal Sciences Lahore, Pakistan for diagnostic purpose. The samples after enrichment in modified tryptose broth (OXOID, Thermo Scientific^{TM}) were cultured on selective and differential media likeMcConkeys agar for isolation. The resultant isolatesshowed pink colonies on McConkeys agarwere suspected as*E. coli*and were further confirmed by specie

specific *16S rRNA* PCR using primers sequence as described by (Magray *et al.* 2011).

Extraction of genomic DNA and its quantification: DNA from purified gram negative bacterial cultures from McConkeys agar was extracted by using the QiagenQIAamp DNA Extraction kit (Qiagen, USA) by spin method following the manufacturer's protocol. DNA quantification was performed with Nanodrop (Thermo Scientific spectrophotometer ND-2000, USA). About 1 µl sample was used to find out the concentration of DNA with the help of Nanodrop.

Confirmation of *E. coli* by *16S rRNA* PCR:

Initially PCR was optimized by using positive control of *E. coli* (ATCC 25922), which amplify the gene sequences of 1183 bp using forward primer 5'-AGAGTTTGATCCTGGCTCAG-3' and reverse primer 5'-CTTGTGCGGGCCCCCGTCAATTC-3'. Each 25 µl of PCR reaction mixture contained 2 µl of upstream primer and2 µl of downstream primer of each gene and DNA fragment, 6.5 µl of nuclease free water, 5 µl of extracted DNA, 5 µlof PCR buffer (100mM), 2.5 µl of dNTPs (200µM) 1.5 µl $MgCl_2$ and 0.5 µl of Taq polymerase. Thermal cycling conditions which were optimized were as follows, initial denaturation at 94ºC for 03 min followed by 30 repeated cycles at 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 01 min and a final extension at 72ºC for 10 min. After performing PCR reaction the PCR product was run on 1.5 per cent agarose gel and electrophoresis was performed. After electrophoresis the gel was visualized on UV transilluminator and was photographed under UV light on gel documentation system (BioRad Corporation, USA).

Antimicrobial resistance: Antimicrobial resistance profiling was conducted by using a total of 15 different antibiotics belonging to five different groups on the basis of their mode of action like protein inhibitors (PI), cell wall inhibitors (CWI), nucleic acid inhibitors (NAI), metabolic inhibitors (MI) and cell membrane inhibitors (CMI) Table # 01. The selection of antibiotics was according to their usage in veterinary and medical sector.The *E. coli* isolates were processed for antimicrobial susceptibility testing according to the Kirby Bauer disk diffusion method and the results were interpreted as per CLSI 2011 criteria (Clinical and Laboratory Standards Institutes) Table # 01.The *E.coli* isolates which were resistant to more than three antibiotics of different groups were categorized as multidrug resistant (MDR).

The data collected was analyzed statistically through correlation using SPSS 18.0 to see correlation of *E. coli* isolates from commercial broiler chicken and backyard poultry with their antibiotic resistance profile.

RESULTS

Out of total n=200 samples, n= 126 *E. coli*isolates (n=70 commercial broilers and n=56 backyard poultry)were confirmed to be *E. coli* via 16SrRNA PCR. The percentage resistance found in *E. coli* isolates recovered from commercial broiler chicken were 28.5%, 28.5%, 57.1%, 28.5%, 21.4% against Protein inhibitor (PI) antibiotic category i.e.Gentamicin (CN), Streptomycin (S), Oxytetracycline (OT), Amikacin (AK), Chloramphenicol (C) respectively, 35.7%, 71.4%, 21.4%, 14.2%,0% against Cell wall inhibitor (CWI) category i.e. Ampicillin (AMP),Amoxycillin (AML), Ceftriaxone (CRO), Ceftiofur (EFT),Cefaclor (CEC) respectively, 42.8%, 28.5%,14.2% against Nucleic acid inhibitor (NAI)category i.e. Levofloxacin (LEV), Ciprofloxacin (CIP), Nalidixic acid (NA), 35.5% against Metabolic inhibitor (MI) category i.e Sulphamethoxazole /Trimethoprim (SXT), 21.4% against Cell membrane inhibitor (CMI) category i.e. Colistinsulphate (CT).

The percentage resistance found in *E. coli* isolates recovered from backyard poultry were 0%, 64.2%, 82.1%, 17.8%, 8.9% against Protein inhibitor (PI) antibiotic category i.e.Gentamicin (CN), Streptomycin (S), Oxytetracycline (OT), Amikacin (AK), Chloramphenicol (C) respectively, 35.7%, 80.3%, 8.9%, 0%, 8%against Cell wall inhibitor (CWI) category i.e. Ampicillin (AMP), Amoxycillin (AML), Ceftriaxone (CRO), Ceftiofur (EFT), Cefaclor (CEC) respectively, 35.7%, 35.7%, 26.7%against Nucleic acid inhibitor (NAI) category i.e. Levofloxacin (LEV), Ciprofloxacin (CIP), Nalidixic acid (NA), 53.5% against Metabolic inhibitor (MI) category Sulphamethoxazole/ Trimethoprim (SXT), 17.8% against Cell membrane inhibitor (CMI) category i.e. Colistinsulphate (CT). The percentage multidrug resistant *E. coli* isolates from commercial broiler chicken and backyard poultry were 64.2% and 53.5%respectively. Statistical analysis reveals that significant association exists between poultry species and antibiotic resistance against OT, CN, S, C, SXT, EFT, CEC and CRO ($p<0.05$) while non significant association ($p>0.05$) was observed between specie of poultry and antibiotic resistance against AK, LEV, CIP, NA, CT, AML and AMP.

Table 1.Name, Code, content, amount of antibiotics in µg and criteria for result interpretation of antibiotic resistance.

Sr. #	Antibiotic Category	Antibiotic Name	Disk Content	Zone Diameter Breakpoints		
				S	I	R
1	Protein inhibitor (PI)	Gentamicin (CN)	10 µg	≥ 15	13-14	≤ 12
		Streptomycin (S)	10 µg	≥ 15	12-14	≤ 11
		Oxytetracycline (OT)	30 µg	≥ 14	11-13	≤ 10
		Amikacin (AK)	30ug	≥17	15-16	≤ 14
		Chloramphenicol (C)	10 µg	≥ 18	13-17	≤ 12
2	Cell wall inhibitor (CWI)	Ampicillin (AMP)	10 µg	≥ 17	14-16	≤ 13
		Amoxycillin (AML)	10ug	≥ 18	14-17	≤ 13
		Ceftriaxone (CRO)	30ug	≥ 23	20-22	≤ 19
		Ceftiofur (EFT)	30ug	8≥	3-4	≤ 2
		Cefaclor (CEC)	30ug	≥18	15-17	≤ 14
3	Nucleic acid inhibitor (NAI)	Levofloxacin (LEV)	5ug	≥ 17	14-17	≤ 13
		Ciprofloxacin (CIP)	5µg	≥ 21	16-20	≤ 15
		Nalidixic acid (NA)	30 µg	≥ 19	14-18	≤ 13
4	Metabolic inhibitor (MI)	Sulphamethoxazole/ Trimethoprim (SXT)	25 µg	≥ 16	11-15	≤ 10
5	Cell membrane inhibitor (CMI)	Colistinsulphate (CT)	25 µg	≥11	9-10	≤ 8

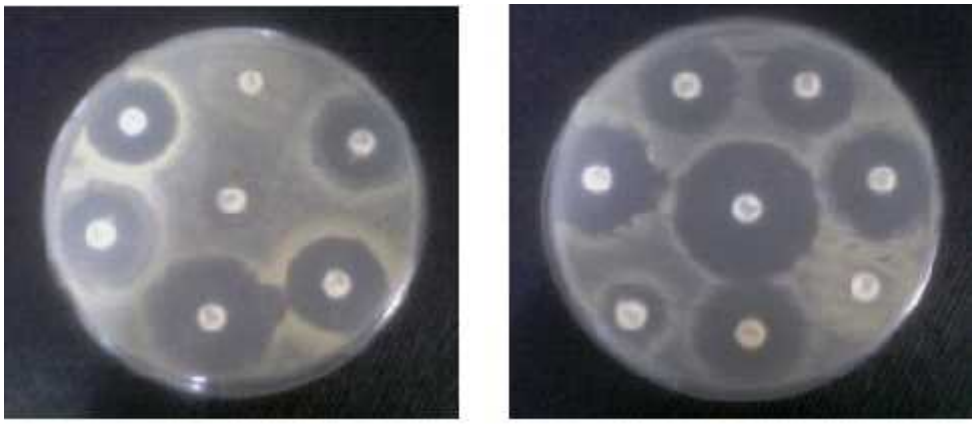

Fig. 1. Plate showing zone of inhibition for various antibiotics against E. *coli*

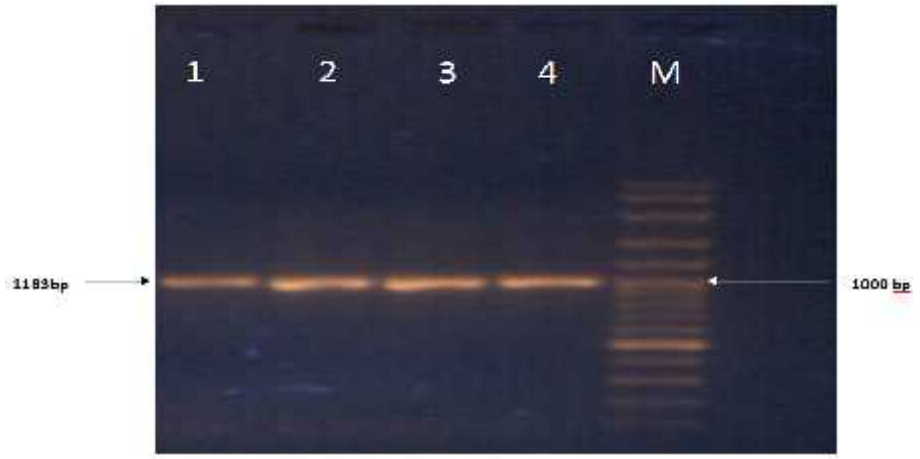

Fig. 2. Ethidium Bromide stained 1.5 per cent agarose gel shows the PCR Amplification of 1183 bp *16S rRNA* gene of*E. coli*

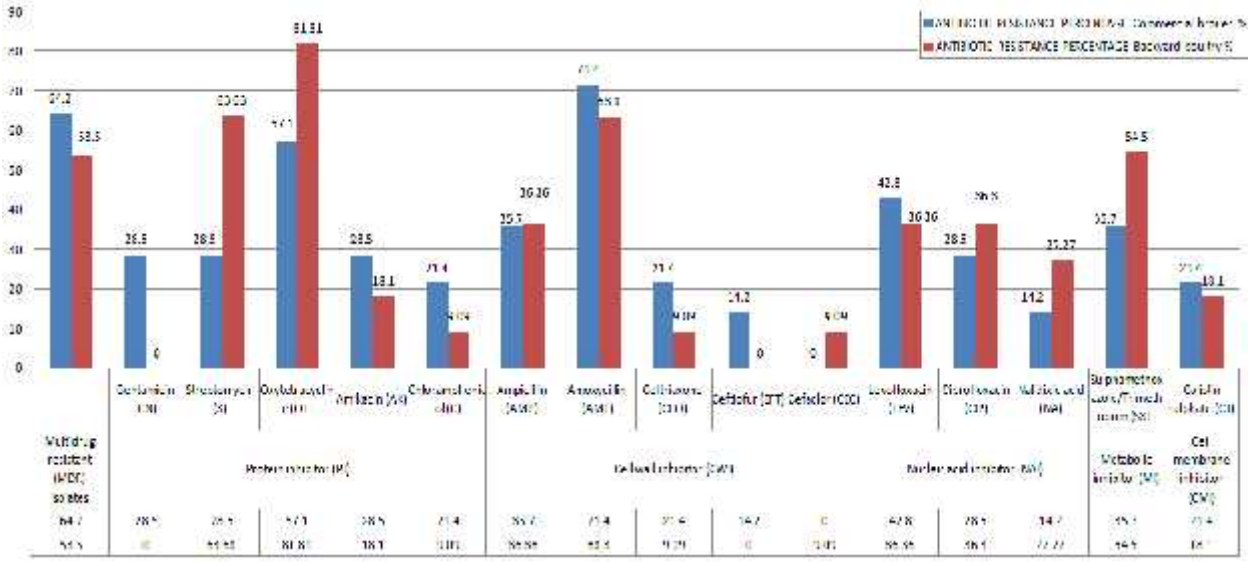

Fig. 3.Comparative Antibiotic Resistance Profile Of The Multidrug Resistant *E. coli* Isolated From Commercial Broiler And Backyard Poultry

DISCUSSION

Antimicrobial resistance study against *E. coli* isolates revealed that highest percentage of resistance was observed in case of protein inhibitor (PI) category of antibiotics i.e. tetracycline. As tetracycline, an older antibiotic which was first time introduced in year 1948. It is routinely used for treatment as well as for growth promotion in commercial feed(Tadesse *et al.* 2012). The percentage antibiotic resistance was observed high in backyard poultry as compared to commercial broiler chicken. This might be due to excessive use of tetracycline in backyard poultry as compared to the commercial broiler chicken because of its cost effectiveness and easy availability. The evidence of tetracycline resistance is not surprising as it is observed against coliforms of animal origin even after a decade when it was no longer used for therapeutic as well as for growth promotion(Langlois *et al.* 1983).

The second most resistant antibiotic was from cell wall inhibitors category (CWI)i.e. amoxicillin. The resistance against this antibiotic was found to be high both in commercial broiler chicken as well as backyard poultry. The main cause as reported by many researchers is considered to be the sub therapeutic administration of this antibiotic(Stapleton *et al.* 1995; Mirzaagha *et al.* 2011). The next level of antibiotic resistance was observed against the metabolic inhibitor (MI) category i.e. sulphamethoxazole/ trimethoprim (SXT), which was introduced in year 1930 (Tadesse *et al.* 2012) both in broiler as well as backyard poultry. The percentage resistance against sulfonamides was found to be high in backyard poultry as compared to the commercial broiler chicken. The main reason of this difference in antibiotic resistance against sulfonamides may be the prolong use of this drug in laying hens against coccidial infections as laying hens are to be kept for long time whereas commercial broiler chicken are mostly kept for 40-45 days so use of sulfa drugs is limited. Although the use of sulfonamides in food animals has been restricted since 1980 due to potential threat of transfer of resistance from food animals to human population, yet the trends in resistance against sulfonamides in *E. coli* are still increasing (Kronvall, 2010).

The percentage resistance against cephalosporin like ceftriaxone, cefaclor and ceftiofur was low both in case of backyard poultry and commercial broilers. Although these drugs were first time introduced in food animals since year 1988, yet in Pakistan the use of cephalosporin is negligible in backyard poultry as compared to commercial broilers. It is assumed that, with the passage of time there is increasing chance of prevalence of resistant *E. coli* against cephalosporins in poultry with increase in the use of these drugs. Similar findings were also recorded by (Sheikh *et al.* 2012; Tadesse *et al.* 2012). This increasing trend of antibiotic resistance against cephalosporin antibiotic give an indication that,due to close community interaction of backyard poultry with human population as well as the use of human left over food for feeding of backyard poultry is considered to be the important cause of this resistance pattern. Multidrug resistant *E. coli* isolates were also recovered from commercial broiler and backyard poultry. There exist strong association amongst the prevalence of oxytetracycline resistance with streptomycin and sulphamethoxazole /trimethoprim resistance in *E. coli* isolates of poultry origin. These findings are in accordance with the fact that the resistance

genes for sulphonamides are reported to be associated with mobile genetic elements, which are very important in dissemination of multidrug resistance genes (Tadesse *et al.* 2012). High levels of associations were generally seen in resistance pattern of *E. coli* against oxytetracycline, ampicillin and streptomycin. Such strong association of resistance pattern in *E. coli* against the same antibiotics was also observed by (Karczmarczyk *et al.* 2011).

The present study revealed that trend of antibiotic resistance has been increasing in backyard poultry as compare to commercial broiler may be due to their interaction with human community.

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