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Detection of antibiotic resistant *Escherichia coli* from poultry meat retail shops in Lahore

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Abstract: Antibiotics are drugs that are used to treat bacterial infections in both humans and animals. These are also used as prophylactic measures as well as growth promoters especially in poultry production. During the last one decade, antibiotic resistance emerged as a global problem drawing attention of the international health agencies. Present study was conducted to detect *Escherichia coli* (*E. coli*) from poultry meat retail shops in Lahore and to further evaluate antibiotic resistance in *E. coli* against different groups of antibiotics. 150 samples were collected from the hands of butchers, knives and their cutting boards with the help of sterile swabs and transferred to the laboratory in icebox. Afterwards, the samples were streaked over the MacConkey agar, pinkish colonies were taken and streaked over EMB agar plates and finally metallic green color colonies were isolated and further identified by the gram staining, catalase, indole, citrate and oxidase tests. Isolated colonies were swabbed over the Müller Hinton agar to check the antibiotic resistance against ampicillin, amoxicillin, tetracycline, gentamicin and ciprofloxacin. In this study, the highest resistance was observed against amoxicillin (80.37%), followed by ampicillin (77.57%), tetracycline (43.93%), ciprofloxacin (27.10%) and gentamicin (18.69%). Results of present study indicated that antibiotic resistance in *E. coli* is increasing day by day owing to the irrational use of these antibiotics.
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Key words: Antibiotic resistance, *E. coli*, Chicken meat, Ampicillin, Tetracycline, Amoxicillin, Gentamicin

1. Introduction

Antibiotics are the agents that are microbicidal and microstatic in action (Leghari *et al.* 2021). Antibiotics are categorized into five major types based on their mechanism of action which includes those inhibiting cell wall synthesis, altering cell membrane structure, inhibiting the synthesis of protein, antimetabolites and inhibiting nucleic acid synthesis (Harakeh *et al*. 2005). In addition to therapeutic use, antibiotics are also being used as a growth promoter or for prophylaxis of various diseases. The irrational use and selection of these antibiotics have caused resistance in many bacteria causing difficulty in treatment (Kolář *et al*. 2001). The antibiotics which are being used in veterinary practices are almost the same as the antibiotics of human use (Aarestrup *et al*. 2008). Also using one class of antibiotics can cause the transfer of resistance against the other classes. Antibiotics are being used without any appropriate dose calculation and even their withdrawal period is not observed by most of the farmers resulting in transferring of antimicrobial residues through food items to humans. Treatment is usually done based on clinical signs rather than the cause of the disease (Gousia *et al*. 2011).

According to the World Health Organization (WHO), food-borne pathogens are causing 30% of the population to suffer disease and causing 2 million deaths in developing countries each year (Shahid *et al*. 2021). Antibiotics are being used for food-producing animals (PFAs) in almost the whole world. Now growth supplements are being used for PFAs for better production and antibiotics are also being used as a growth promoter. There is an estimation about the annual consumption of antibiotics as 45mg/kg for cattle, 48mg/kg for chicken and 172mg/kg for pigs in 2010 and this may increase by 67% in 2030 (Rahman and Mohsin 2019). Even antibiotic residues have been recorded in milk in some studies which indicated that 36.50% of commercial milk samples were positive for the beta-lactams antibiotic residues (Khaskheli *et al*. 2008). Also in some other studies, it has been

suggested that high concentrations of these antibiotic residues have been found in meat, eggs, and milk in various parts of Pakistan.

The use of antimicrobial agents in food-producing animals is unregulated in Pakistan and there are no estimates about its annual usage even though antimicrobial resistant has been identified as a major concern in Pakistan (Mohsin *et al.* 2017). It is very difficult to find exact data regarding the use of antibiotics for treatment, growth promoter and prevention in food-producing animals in Pakistan. When we see trends of practice at commercial and domestic level then we see that most of the antibiotic usage is being done for prevention and as a growth promoter. Resistance bacteria can be transferred to the meat products due to the unhygienic slaughtering process from the intestinal and fecal contaminants and also due to improper handling of the tissues during the process (Schroeder *et al.* 2003). Meat is consumed by most of the people worldwide. According to FAO 32.4kg pork, 21.9kg poultry, 16.1 kg beef is consumed annually in Europe per person. Because of this higher consumption rates its quality is a major public health concern (Skočková *et al.* 2015). As antibiotics are being used in animal production also so the bacteria which originate from the food could carry and transfer the resistance genes to the human (Hammerum and Heuer 2009).

These resistant bacteria could also act as a reservoir and could transfer these genes to commensal as well as pathogenic bacteria in the human digestive tract (Álvarez-Fernández *et al.* 2013). Antibiotic resistance can be considered as one of the greatest threats for the human population (Shahid *et al.* 2021). Annually 2 million or even more people in the United States get infected with resistant bacteria which cause the death of 23000 people directly due to antibiotic resistance. It anticipated that by 2050, deaths of more than 10 million people worldwide will be due to antibiotic resistance (Marquardt and Li 2018). New antibiotics are not being developed at the same rate as antibiotic resistance is increasing. Contaminated and undercooked raw meat has been seen as source of most common and important human infections due to foodborne pathogens which mostly include *Staphylococcus aureus*, *Escherichia Coli* and *Salmonella*. And these are the agents who are mostly involved in cases of food poisoning due to contaminated meat in developing countries and also in the USA. It is also observed that food contaminated with antibiotic resistant bacteria is a huge public health concern in most of the countries due to continuous circulation of these resistant strains in the environment (Gwida and El-Gohary 2015). *Escherichia coli (E. coli)* served as the candidate vehicle for the transfer of these resistance determinants to the other agents because it is part of the normal flora of the animals and humans.

There are also other food-borne agents who carry the resistance genes and causing their transfer to other bacteria. *E. coli* are also used as an indicator for assessing the presence of other enteric organisms (Altalhi and Hassan 2009). When common, *E. coli* infections will be resistant to antibiotic especially showing multidrug resistance, and then there will be many complications for the treatment of patients. It is not necessary that if resistant *E. coli* is ingested it will have immediate effects; there is also possibility that they may transfer these resistant genes to more fatal bacteria which can cause many complications and also to other bacteria that are present in the gut. So it will be very difficult in future to treat bacterial infections (Davis *et al.* 2018). This study was conducted because most of the people in Pakistan consume chicken meat which is slaughtered in small retail shops where the hygienic conditions are very poor. This can increase the chance of bacterial contamination which can also transfer the antibiotic resistance to humans, hence, making the treatment of most of the infectious diseases ineffective.

2. Materials and methods
2.1. Sample collection
A total of 150 samples were collected from retail chicken meat shops situated in various regions of Lahore e.g. Johar town, Islampura, Harbanspura, Taj Bagh and Mughal pura. The swab samples were taken from the hands of the butchers, knives and cutting boards. All the samples were collected and processed separately.

2.2. Samples preparation and processing
All the samples were collected with pre-soaked sterile nutrient broth. The collected samples were transferred to the laboratory on the same day maintaining the cold chain. Samples were kept at 4 °C until further processing. The nutrient broth with samples was kept in the incubator for 18 to 24 hours for the nourishment of bacteria at 37 °C.

2.3. Isolation and identification
A loopful of samples was inoculated over the MacConkey agar (Oxoid, Basingstoke, Hampshire, United Kingdom) and incubated at 37 °C for 24-48 hours. After incubation, presumptive *E. coli* colonies showing pink color were collected and inoculated over Eosin Methylene Blue agar (Oxoid, Basingstoke, Hampshire, United Kingdom) and incubated for 24-48 hours at 37 °C. After that, colonies showing metallic green color were further collected and identified using gram staining, catalase, oxidase and indole production (Vos *et al.* 2011).

2.3.1. Gram Staining

For this presumptive metallic green color, *E. coli* colonies were taken with a sterile platinum loop from the EMB agar plate. These colonies were then mixed with the drop of water already placed on the glass slide and a smear was made with the help of loop over the glass slide. It was then allowed to be air-dried. After that crystal violet was put for 1 minute which was then washed with tap water then gram iodine was put for 1 minute which was then washed with tap water after that 70% alcohol was added over the glass slide for 15-30 seconds which was washed with tap water. And in the end, safranin was added for 1 minute and washed with tap water. Afterward, the slide was air-dried and after that, the slide was observed over the compound microscope.

2.3.2. Biochemical profile

The suspected colonies were also observed by oxidase, catalase, citrate and indole production test.

2.3.2.1. Catalase test

For this one drop of 2-3% of hydrogen peroxide was mixed with the presumptive *E. coli* colonies over the glass slide. And the positive catalase was identified as the formation of bubbles and vice versa.

2.3.2.2. Oxidase test

Tetramethyl-p-phenylenedimine dihydrochloride is colorless redox reagent. There is a chromogenic compound in it. And if their color changed into blue then it is positive and if no color change then it was negative.

2.3.2.3. Indole test

Tryptophan broth was prepared and was put in the sterile test tubes. Presumptive *E. coli* colonies were then added in these test tubes and incubated at 37 °C for 24 hours. The few drops of Kovac's reagent were added in this. Then red color ring was identified as positive and no change in color was considered as negative.

2.3.2.4. Citrate utilization test

In this Simmons's citrate agar (Oxoid, Basingstoke, Hampshire, United Kingdom) was used and presumptive *E. coli* colonies were streaked over the agar. And incubation was done at 37 °C for 24 hours. Then change in color was seen and observed as positive and no change in color was seen as negative.

2.4. Antimicrobial susceptibility test

Antibiotic susceptibility testing was performed to see the resistance pattern of different antibiotics used in this study.

2.4.1. Antibiotics disc

The antibiotics used in this experiment included two antibiotics from beta-lactams class (ampicillin, amoxicillin), one from aminoglycosides (gentamicin), one from tetracyclines (tetracycline) and one from the quinolones (ciprofloxacin). All the antibiotics used in this experiment were from (Oxoid, Basingstoke, Hampshire, United Kingdom).

Table 1: Antibiotics used in experiment

Antibiotic class	Antibiotic	Quantity (μg)
Beta lactams	Ampicillin	10
	Amoxicillin	30
Aminoglycosides	Gentamycin	10
Tetracyclines	Tetracycline	30
Quinolones	Ciprofloxacin	5

The quantity for the antibiotics was according to the guidelines by Clinical and Laboratory Standards Institute (2019), which were ampicillin (10 μg), amoxicillin (30 μg), tetracycline (30 μg), gentamicin (10 μg) and ciprofloxacin (5μg).

2.4.2. Preparation of inoculum

The *E. coli* isolates were inoculated in 5 ml of phosphate buffer solution (PBS) in sterile test tubes which was then suspended for 2-6 hours at 37°C. We took 1 ml PBS from the already suspended PBS tube into a new sterile tube to make a new standard inoculum by comparing it to the 0.5 McFarland turbidity standards.

2.4.3. Sensitivity test

Already prepared inoculum was inoculated on Müller-Hinton agar (Oxoid, Basingstoke, Hampshire, United Kingdom) by swabbing throughout its surface. The Kirby-Bauer disk diffusion method was performed to check the antibiotic resistance in *E. coli* against before mentioned antibiotics.

2.4.4. Interpretation of results

Zone of inhibition was measured for each of the antibiotic and was compared with the CLSI (2019) standards. All the isolates were then declared sensitive, intermediate resistant and complete resistant according to CLSI (Table 2).

Table 2: Standards of antibiotics from CLSI M100 ED29

Antibiotics	Antibiotic Disc (*μg*)	Sensitive	Intermediate	Resistant
Ampicillin	10	≥17	14-16	13≤
Amoxicillin	30	≥17	--	16≤
Tetracycline	30	≥15	12-14	11≤
Gentamicin	10	≥15	13-14	12≤
Ciprofloxacin	5	≥26	22-25	21≤

3. Results

3.1. Isolation and identification of *E. coli*

A total of 150 swab samples were collected from the retail poultry meat shops in Lahore. Out of total isolates, positive isolates were 107(71.33%) and negative isolates were 43(28.67%).
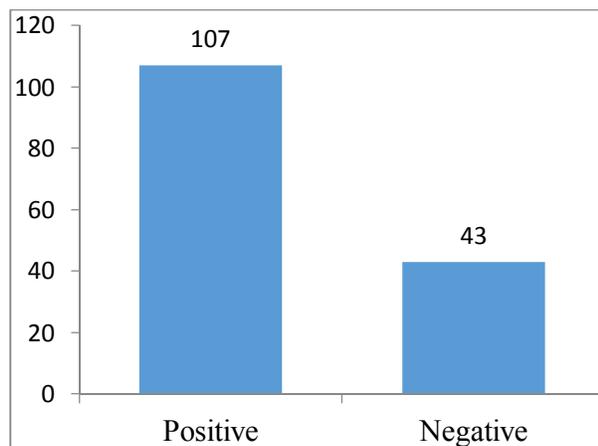

Figure 1A: Total Positive and negative isolates (n=150)

Out of total, isolates collected from the hands of butchers were 75. From these 75, *E. coli* was isolated from 48 (64%) isolates.
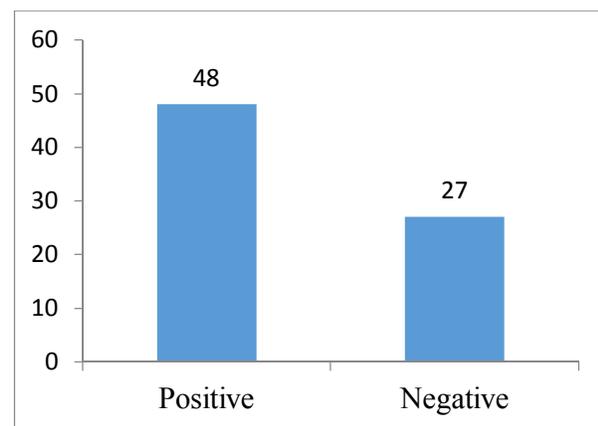

Figure 1B: Positive and negative isolates collected from hands of butchers (n=75)

Out of total, isolates collected from the knives and cutting boards of butchers were 75. From these 75, *E. coli* was isolated from 59(78.67%) isolates.
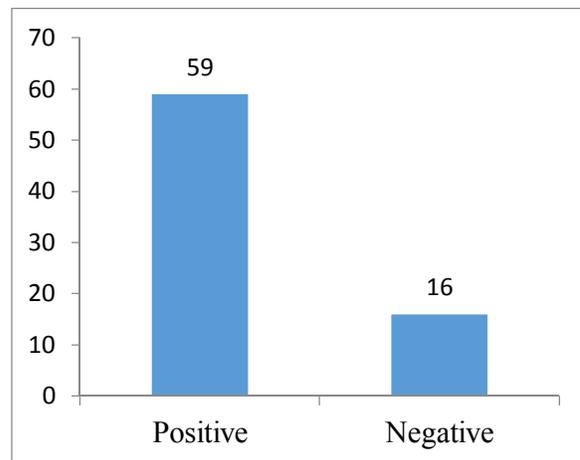

Figure 1C: Positive and negative isolates collected from knives and cutting boards of butchers (n=75)

3.2. Antibiotic resistance pattern

In figure 2A, the overall resistance was highest in amoxicillin (80.37%), followed by ampicillin (77.57%), tetracycline (43.93%), ciprofloxacin (27.10%) and gentamicin (18.69%).

Out of 107 isolates, 80.37% isolates showed complete resistance against amoxicillin, while 19.63% isolates were sensitive. In the case of ampicillin, 77.57% showed complete resistance, whereas 9.35% isolates showed intermediate resistance and 13.10% were sensitive to the drug. Out of the total, 43.93% isolates showed complete resistance against tetracycline; whereas 23.36% showed intermediate resistance and 32.71% were sensitive. Complete resistance against ciprofloxacin was showed by 27.10% of isolates; whereas 28.04% isolates showed intermediate resistance and 44.86% isolates were sensitive. Gentamicin was least resistant of all antibiotics used in this experiment which was just 18.69%, while 77.57% of *E. coli* isolates were sensitive to this drug.

3.2.1. Antibiotic resistance pattern of *E. coli* isolated from hands of butchers

Out of total 75, *E. coli* was isolated from 48 samples. In figure 2B, out of 48 positive *E. coli* isolates, 37 (77.08%) were complete resistant against

ampicillin and only 6 (12.5%) were sensitive while 5(10.42%) showed intermediate resistance. Complete resistance against amoxicillin was showed by 39 (81.25%), while 9 (18.75%) were sensitive to the use of amoxicillin. Complete resistance against tetracycline was showed by 23 (47.92%) isolates, while 11 (22.92%) isolates showed intermediate resistance, however, tetracycline sensitive isolates were only 14 (29.17%). Complete resistance against gentamicin was showed by 12 (25%) isolates while 2 (4.17%) isolates showed intermediate resistance. Gentamicin sensitive isolates were 34 (70.83%). Complete resistance against ciprofloxacin was showed by 13 (27.08%), while 14 (29.17%) isolates showed intermediate resistance. Isolates sensitive to the use of ciprofloxacin were 21 (43.75%).

3.2.2. Antibiotic resistant pattern of *E. coli* isolated from knives and cutting boards at retail meat shops in Lahore

Out of total 75, *E. coli* was isolated from 59 isolates and the remaining 16 were negative for *E. coli.* Out of 59 positive *E. coli* isolates, 46 (77.96%) were complete resistant against ampicillin and only 8 (13.56%) were sensitive, while 5(8.47%) showed intermediate resistance. Complete resistance against amoxicillin was showed by 47 (79.66%), while 12 (20.34%) were sensitive to amoxicillin. Complete resistance against tetracycline was showed by 24 (40.68%) isolates, while 14 (23.73%) isolates showed intermediate resistance. Tetracycline sensitive isolates were 21 (35.59%). Complete resistance against gentamicin was showed by 8 (13.56%) isolates, while 2 (3.39%) isolates showed intermediate resistance. Gentamicin sensitive isolates were 49 (83.05%). Complete resistance against ciprofloxacin was showed by 16 (27.12%), while 16 (27.12%) isolates showed intermediate resistance. Isolates sensitive to the use of ciprofloxacin were 27 (45.76%).
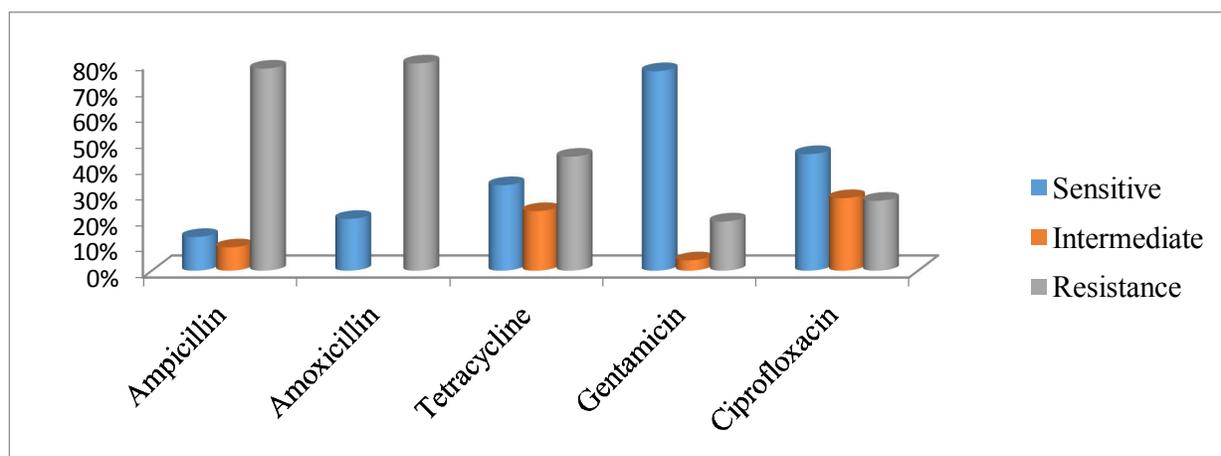

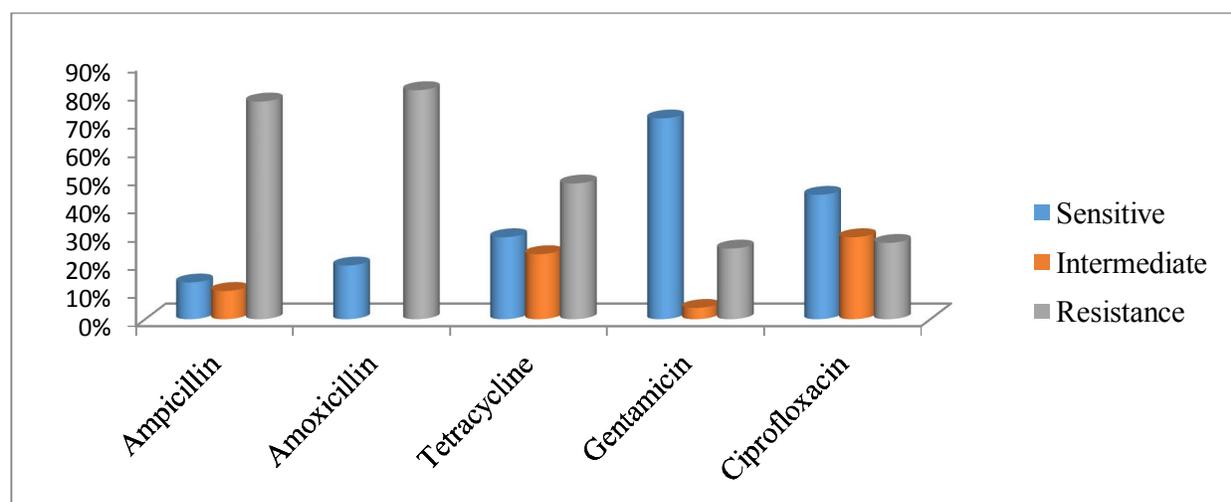**Figure 2A: Antibiotic resistance pattern of *E. coli* isolated from retail meat shops in Lahore**

Figure 2B: Antibiotic resistance pattern of *E.coli* isolated from hands of butchers at retail meat shops in Lahore
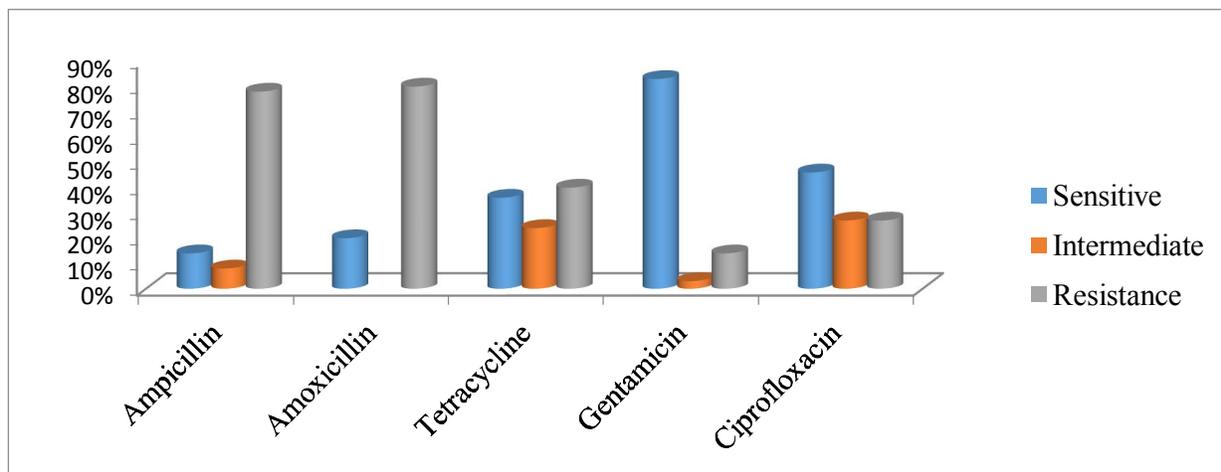
Figure 2C: Antibiotic resistance pattern of *E. coli* isolated from knives and cutting boards at retail meat shops in Lahore

Out of 107 positive *E. coli* isolates, 53(49.53%) showed resistance against 2 antibiotics. In Table 3, resistance against ampicillin and amoxicillin was observed in 29(27.10%) isolates. Against ampicillin and tetracycline, it was seen in 8(7.48%) isolates collectively from hands, knives and cutting boards of the butcher. Resistance against amoxicillin and tetracycline was observed in 4(3.74%) samples collectively. Resistance against amoxicillin and ciprofloxacin was observed in 5(4.67%) samples collectively. Resistance against tetracycline and ciprofloxacin was recorded in 2(1.87) isolates. Resistance against amoxicillin and gentamicin was observed in 2(1.87%) isolates. Resistance against gentamicin and ciprofloxacin, gentamicin and tetracycline also ampicillin and gentamicin was observed in 1(0.93%) sample each.

Table 3: Antibiotic resistance profile of *E.coli* (at least two antibiotics)

Antibiotics	Sample count (%)
AML+CIP	5(4.67%)
AMP+AML	29(27.10%)
AML+TE	4(3.74%)
CN+CIP	1(0.93%)
AMP+TE	8(7.48%)
CN+TE	1(0.93%)
TE+CIP	2(1.87%)
AMP+CN	1(0.93%)
AML+CN	2(1.87%)

AMP=Ampicillin, AML=Amoxicillin, TE=tetracycline, CN=Gentamicin, CIP=Ciprofloxacin

At least 3 antibiotics were showing resistance in 45(42.06%) isolates of *E. coli* out of total 107. Resistance against ampicillin, amoxicillin and tetracycline was seen in 15(14.01%) samples. Resistance against ampicillin, tetracycline and ciprofloxacin was observed in 7(6.54%) samples. Ampicillin, amoxicillin and gentamicin showed resistance in 9(8.41%) samples collectively. Resistance against ampicillin, amoxicillin and ciprofloxacin was recorded in 8(7.84%) isolates. Resistance against amoxicillin, gentamicin and ciprofloxacin was observed in 2 samples. Ampicillin, ciprofloxacin and tetracycline were resistant against 1(0.93%) isolate collectively. In other 2(1.87%) isolates resistance was recorded against ampicillin, tetracycline and gentamicin collectively.

Table 4: Antibiotic resistance profile of *E.coli* (at least three antibiotics)

Antibiotics	Sample count
AMP+AML+CN	9(8.41%)
AMP+AML+TE	15(14.01%)
AML+CN+CIP	2(1.87%)
AMP+CIP+TE	1(0.93%)
AMP+AML+CIP	8(7.48%)
AMP+TE+CN	2(1.87%)
AML+TE+CIP	7(6.54%)
AMP+TE+CIP	1(0.93%)

Resistance against at least 4 antibiotics was recorded in 5 (4.67%) isolates out of total 107.

Table 5: Antibiotic resistance profile of *E.coli* (at least four antibiotics)

Antibiotics	Sample count
AMP+AML+TE+CN	3(2.80%)
AMP+AML+TE+CIP	2(1.87%)

4. Discussion

Antibiotic resistance has been seen worldwide as an emerging problem in veterinary medicine as well as in humans including developed and underdeveloped countries (Caniça *et al*. 2019). It is also well observed that most of the environmental and food sources contain bacteria which also contain these resistant genes, due to maximum use of antibiotic in veterinary medicine, food animal production and also in humans (Anderson *et al*. 2003). The antibiotics which are being used in veterinary practices are almost the same as the antibiotics of human use (Aarestrup *et al*. 2008). Also using one class of antibiotics can cause the transfer of resistance against the other class. Antibiotics are being used without any appropriate dose calculation and even their withdrawal period is not observed by the farmers. Treatment is done at only clinical sign basis rather than against the actual cause of the disease (Gousia *et al*. 2011). Resistance bacteria can be transferred to the meat products due to the unhygienic slaughtering process from the intestinal and fecal contaminants and also due to improper handling of the tissues during the process (Schroeder *et al*. 2003).

In this study, *E. coli* have been isolated from 107 samples out of the total 150. This high level of contamination shows the poor hygienic conditions of the chicken retail shops. A total of 48 *E. coli* isolates were isolated from the hands of the butcher at chicken retail shops and 59 *E. coli* isolates were isolated from the knives and cutting board of the butcher. This variation may be the results of the sampling time because sampling was done at different hours of the day due to convenience. Some of the butchers had clean hands and sampling was also done during early hours of the morning at some chicken retail shops from the knives and cutting board when the bacterial load was very less. On the other hand, bacterial colonies were isolated from the total 150 samples but only 107 were identified as *E. coli* and the remaining were showing different characteristics than the *E. coli* and further not identified. Antibiotic resistance in *E. coli* is a high concern because this gram negative bacteria is most common in humans, animals and environment, which is also cause of urinary tract infection and also causes community and hospital-acquired bacteremia (Salvadori *et al*. 2004) and causing many cases of diarrhea (Kaper *et al*. 2004). And it is also seen that not only this resistant *E. coli* can transfer these resistant genes to other *E. coli* strains but also these genes can be transferred to other strains of bacteria in the gastrointestinal tract (Österblad *et al*. 2000).

All the positive *E. coli* isolates were showing resistance to at least one of the antibiotics. Out of this 82(76.63%) were showing resistance to at least two

antibiotics. Multidrug resistance was seen in 37(34.58%) isolates out of total. This shows the high level of antibiotics resistance which is prevailing and being transferred through the chicken meat shops. This high level of resistance is due to the improper and uncalculated use of antibiotics in poultry production. Also, the sampling was done in mostly summer season which is hot and humid and favors the growth of bacteria (Vangchhia *et al*. 2018), which could indirectly increase the transfer of resistance to the other strains. Presence of the *E. coli* at retail meat shops shows very poor sanitary condition which is a risk for consumer health. It is seen that by proper cooking or heating most of the bacteria die due to high temperature but the condition like undercooking, poor handler hygiene, cross-contamination between cooked and raw food can distribute these resistant strains (Martínez-Vázquez *et al*. 2018).

This study was done to see the antibiotic resistance in *E. coli* against the most commonly used antibiotic in the poultry and for this five different antibiotics was selected. The highest resistance was against amoxicillin (80.37%) and the lowest resistance was against gentamicin (18.69%). A study like this was also conducted in Malaysia which also shows resistance against ampicillin 57.14% (Tan *et al*. 2014) is lower than our study which is (77.57%). This shows that antibiotic resistance is increasing even in most common bacteria like *E. coli* due to excessive use of antibiotics in the poultry farming practice. In another study in Mexico, resistance against amoxicillin/clavulanic acid was recorded at 54.4% (Martínez-Vázquez *et al*. 2018) but in our study, it showed the value of 80.37%. That is so much higher than the previous study which is increasing day by day due to the uncontrolled use of antibiotics.

Antibiotics are being used as a growth promoter in poultry feed (Salim *et al*. 2018). So the risk of antibiotic resistance is increasing. When antibiotics are used as growth promoter then normal flora of the gut of the bird or animal can be exposed to that antibiotic and the chances of the antibiotic resistance against that antibiotic may increase (Xu *et al*. 2020). Also in Pakistan due to poor management, the cases of early chick mortality are high on most of the commercial farms. To prevent early chick mortality antibiotics are used in the feed of the bird or in drinking water, so the birds are exposed to antibiotics from the early stages of their growth which may also be the cause of high antibiotic resistance in this study(Akbar *et al*. 2014).

A study was conducted in Australia in which the resistance against tetracycline was observed as 39% (Vangchhia *et al*. 2018) As we compared this to our study in which the resistance against tetracycline was recorded as 43.93%. This showed an increase in antibiotic resistance in our study. This may be due to

irregular use of antibiotics in poultry production practices in Pakistan, Also oxytetracycline which was a member of tetracycline group has been approved to be used as growth promoter (Chopra and Roberts 2001), so it was being used in poultry feed which could be a cause of increase in resistance against tetracycline. The resistance against tetracycline was less in the previous study in Australia because the trend of antibiotic usage was somehow controlled as compared to Pakistan because there was not good check and balance about antibiotics usage and also lack of knowledge about threats of antibiotic resistance (Mohsin 2019).

The demand for chicken meat is increasing day by day locally and globally and due to the high demand for meat, the density of the chicken at farms is high (Kumar *et al*. 2019). Due to high density, there are many chances of disease in the farms. So to prevent this antibiotics are used even in the final stages of the growth and the antibiotics withdrawal period is not followed which causes antibiotic resistance production in the gut flora of the bird (Sajid *et al*. 2016). In these high crowded farms, sub-therapeutic level of antibiotics are given so that the diseases should be prevented, which causes antibiotic resistance against that antibiotic (Muaz *et al*. 2018)

A study in Bangladesh was conducted to check the antimicrobial resistance. In this study, it was recorded that all the isolates of *E. coli* were sensitive to gentamicin (Sarker *et al*. 2019) but when we observed in our study it showed that 18.69% of isolates were resistant to gentamicin and 77.57% were sensitive to the use of gentamicin. This showed that it was higher in our study which may be the result of poor antibiotics practices. The resistance against gentamicin was the lowest among all antibiotics used in this study. The possible reason could be the form of antibiotics in which this came. Because gentamicin mostly comes in injectable form, so it could be very difficult to administer gentamicin to the whole poultry flock. Most of the farmers in Pakistan are illiterate and they do not have much knowledge of the antibiotic withdrawal period and also about the proper dosage of antibiotics in poultry production practices. This causes the bacteria to produce antibiotic resistance against these antibiotics. This resistance is not bound to those bacteria only as this can transfer from one bacterium to another or even other species of bacteria which was also cause of high antibiotic resistance in this study.

The resistance against ciprofloxacin was also less (27.10%) among all the antibiotics used in this study except gentamicin. Similar results against ciprofloxacin (27.02%) were also observed in another study in Karachi (Ali *et al*. 2010). Another study was conducted in which the resistance against ciprofloxacin was (85%) (Hassan *et al*. 2011), this was

because the sampling was done from the humans which were suffering from the urinary tract infection in a tertiary care hospital and receiving these antibiotics but in our study sampling source was different and possible reason for this could be cost of ciprofloxacin. Because most of the poultry farms work on the cost effective basis so they prefer low priced antibiotics to increase their profit margins (Suresh *et al*. 2018), that's why resistance against ciprofloxacin has not developed that much as other antibiotics used in this study.

5. Conclusion

In this study out of 150 samples, *E. coli* was isolated from 107 samples and highest resistance was observed against amoxicillin (80.37%) while it was lowest (18.69%) against gentamicin. In this study almost all the isolates (*E. coli*) were resistant to at least 1 antibiotic; these can serve as a pool for resistance transfer to other infectious bacteria that could make the treatment of infections more difficult. Also, these retail meat shops could serve as a potential cause for the transfer of antibiotic resistance to humans as they are the ultimate consumer. Proper washing of meat should be done to avoid any contamination with potential resistant bacteria. The government should take effective measures to stop the irrational use of antibiotics in poultry feed. Studies like these should be carried out on a more extensive level and also the genes responsible for the resistance should be identified by using more recent techniques.

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