Original Article

Microbiological Profile and Susceptibility Pattern of Enteric Organisms in Raw Broiler Chicken Meat from Abattoirs of Lahore, Pakistan

Estimate Number of Enteric Pathogens in Raw Chicken

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ABSTRACT

Objective: This study aimed to estimate number of enteric pathogens in raw chicken meat including surfaces of breast and cloacal region. Moreover, antibiotics susceptibility pattern of organisms isolated from thigh meat were also observed.

Study Design: A Descriptive, cross-sectional study.

Place and Duration of Study: This study was conducted at the Department of Microbiology, University of Health Sciences (UHS) Lahore. December 2021 to April 2022.

Methods: About 95 raw broiler chicken thigh meat samples from abattoirs of different areas of Lahore were collected. Swabs from chicken breast and cloacal region were also taken to estimate the microbes present on their surface.

Results: The mean Aerobic Plate Count was 4.75+1.18 log CFU/g. Out of 95 chicken samples, thigh meat had E. coli (n= 67), Proteus spp., (n=15), Enterobacter spp., (n=6), Citrobacter spp., (n=6) and S. aureus (n=3). From breast swab, E. coli (n= 69), Proteus spp., (n=16), Enterobacter spp., (n=9), Yersinia spp., (n=1), Citrobacter spp., (n=4), S. aureus (n=9) and Klebsiella spp., (n=6) were retrieved. Cloacal swabs revealed E. coli (n= 59), Proteus spp., (n=67), Enterobacter spp., (n=5), Yersinia spp., (n=1) and Citrobacter spp., (n=2). All the isolates were resistant to ampicillin.

Conclusion: Various Enterobacteriaceae species especially E. coli are common in broiler chickens at abattoirs. The presence of multi drug resistant strains of these enteric organisms is an important finding of this study.

Key Words: Antibiotics, Cross contamination, E. coli, Enterobacteriaceae, Multidrug resistant bacteria, Poultry meat, Raw Chicken

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INTRODUCTION

Chicken meat is preferred due to its low fat and cholesterol content. This quality makes it a healthy food choice, which is not only readily available but also a rich and cheaper source of proteins[1]. The consumption of poultry meat is therefore increasing worldwide. An increase in demand of chicken meat should not compromise its hygiene because contaminated or raw meat can possibly transmit food-borne pathogens[2]. Food borne diseases are one of the leading causes of mortality and infections, especially in developing countries. Poultry meat is mostly found to harbor various enteric pathogens such as Salmonella, Campylobacter, S. aureus, E. coli and Listeria. Some members of Enterobacteriaceae family e.g. Escherichia coli, Salmonella, Klebsiella and Proteus are known to cause putrefaction of chicken meat and other food products[3]. The improper use of antibiotics for disease prevention and rapid growth promotion in broiler chickens is the leading source of development of bacterial resistance in them[4]. The intestinal bacteria, thus plays a vital role as vehicle of drug resistance genes which may be transmitted to other clinically important bacteria[5]. An amount of 10g meat sample (thigh) was thoroughly minced. It was diluted in 90ml (w/v) of buffered peptone water to achieve 1:10 dilution. A volume of one ml from meat solution was serially diluted to 1:10 dilution[6]. An amount of 0.1ml or 100µl was taken from TT1, TT3 and TT5 respectively and added to nutrient agar plates. The

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solution was spread evenly with the help of glass L-shaped spreader. The plates were incubated at 35-37°C for 24-48 hours. After incubation, colonies were counted on a colony counter and colony forming unit/gram (CFU/g) was calculated[7].

METHODS

The study employed a descriptive, go-sectional layout carried out on the University of Health Sciences, Lahore, from December 2021 to April 2022. Convenient sampling become used to gather ninety five raw broiler bird thigh meat samples from various abattoirs in Lahore. Additionally, swabs from bird breast and cloacal location were taken for microbial estimation. The meat samples have been processed by way of dilution in buffered peptone water, observed through serial dilution and plating on nutrient agar. Identification of Salmonella and Campylobacter became accomplished the usage of selective media and biochemical exams. Antimicrobial susceptibility checking out became carried out the usage of the Kirby Bauer Disk Diffusion approach, following CLSI breakpoints. Statistical analysis employed One Way ANOVA.

Data collection: The records series for this examine involved obtaining 95 raw broiler chicken thigh meat samples from numerous abattoirs in Lahore thru convenient sampling. Swabs from the chicken breast and cloacal region have been additionally collected to assess microbial presence. A meticulous procedure blanketed mincing 10g of thigh meat, diluting it in buffered peptone water, and serial dilution for next plating on nutrient agar. The identification and locality of the sampled areas have been duly stated. Furthermore, the take a look at incorporated the identity of Salmonella and Campylobacter via selective media and biochemical assessments. The antimicrobial susceptibility of isolated organisms turned into assessed using the Kirby Bauer Disk Diffusion approach.

Statistical analysis: Data was expressed as mean and standard deviation. Bacterial counts were compared by One Way ANOVA test using SPSS Software 24.0 to determine difference in group means at P value ≤ 0.05.

RESULTS

According to Table 1, out of 95 raw broiler chicken samples, 39.0% had APC <4 log CFU/g, 22.1 % had >4 to <5 log CFU/g, 26.3 % had >5 to <6 log CFU/g, and 12.6% had >6 log CFU/g. The average APC was 4.751+1.1811 log CFU/g. This demonstrates that most samples met meat hygiene standards. This research detected no Salmonella or Campylobacter. Figure 1 shows that E. coli was recovered from 67 (70.5%) thigh meat samples, whereas Proteus, Enterobacter, Citrobacter, and S. aureus were isolated from 15 (15.8%), 6 (6.3%), 6 (6.3%), and 3 (3.2%) samples. Table 2 shows chicken flesh, breast, and cloacal swabs' identified enteric microbes. E. coli was identified from 59 (62.1%) cloacal samples, whereas Proteus Spp., Enterobacter, Yersinia spp., and Citrobacter were isolated from 67 (70.5%), 5 (5.3%), 1 (1.0%), and 2 (2.1%) samples E. coli was recovered from 69 (72.6%) of 95 breast swabs, whereas Proteus, Enterobacter, Yersinia, Citrobacter, S. aureus, and Klebsiella were isolated from 16 (16.8%), 9 (9.5%), 1 (1.0%), 4 (4.2%), 9 (9.5%), and 6 (6.3%).

Using ANOVA, significant findings (P < 0.05) were seen for E. coli, Proteus Spp., and Enterobacter spp., whereas Citrobacter and S.aureus exhibited negligible effects (P > 0.05).

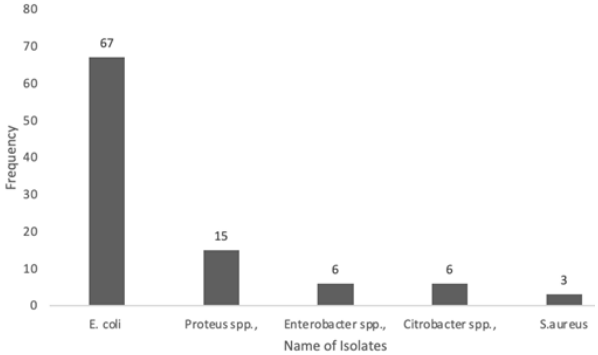

Figure No. 1: Spp., of bacteria isolated from thigh meat (n=95)

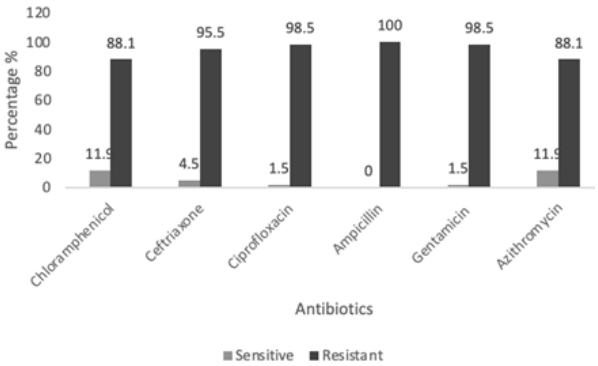

Figure No. 2: Antibiotic susceptibility pattern of E. coli (n= 67)

Table No. 1: Aerobic plate count from chicken thigh meat

Log CFU/g	Number	Percentage%
<4	37	39.0
>4 to <5	21	22.1
>5 to <6	25	26.3
>6*	12	12.6
Total	95	100.0
Mean + SD	4.751 + 1.1811	

*>6 log CFU/g depicts increased chance of spoilage of meat.

Table No. 2: Bacteria isolated from different types of samples

Sampling type	No. of samples	E. coli	Proteus spp.	Enterobacter spp.,	Yersinia spp.,	Citrobacter spp.,	S. aureus	Klebsiella spp.,
Thigh meat	95	67	15	6	0	6	3	0
Cloacal swab	95	59	67	5	1	2	0	0
Breast swab	95	69	16	9	1	4	9	6
Total	285	195	98	20	2	12	12	6

Table No.3: Frequency of antibiotic-resistant enteric strains isolated from chicken thigh meat

Bacterial strains	Antibiotics resistance %					
	Chloramphenicol C30µg	Ceftriaxone CRO30µg	Ciprofloxacin CIP5µg	Ampicillin AMP10µg	Gentamicin CN10µg	Azithromycin AZM15µg
E.coli (n=67)	59(88.1%)	64(95.5%)	66(98.5%)	67(100%)	66(98.5%)	59(88.1%)
Proteus spp., (n=15)	9(60.0%)	10(66.7%)	11(73.3%)	15(100%)	10(66.7%)	15(100%)
Enterobacter spp., (n= 6)	6(100%)	6(100%)	6(100%)	6(100%)	6(100%)	6(100%)
Citrobacter spp., (n=6)	5(83.3%)	5(83.3%)	6(100%)	6(100%)	6(100%)	6(100%)
S.aureus (n=3)	2(66.6%)	1(33.3%)	3(100%)	3(100%)	3(100%)	1(33.3%)

DISCUSSION

Aerobic plate count (APC) measures meat microbiology. European Union Standards and British meat processors association recommend a raw meat aerobic plate count below 106 CFU/g or cm2. Table 1 aerobic plate count results matched a local investigation that found 9 of 45 chicken samples with APC >6 log CFU Other Karachi and Lahore investigations found higher APC values All of these data emphasise the need to enhance slaughtering techniques and abattoir hygiene[8,9]. Human flora, slaughtering personnel's hand hygiene, slaughtering table and instrument flora, slaughterhouse temperature and humidity all affect bacterial burden during processing. E. coli was the most common microbe in this investigation. 70% of thigh meat samples had E. coli[10]. One local study found 85% E. coli in raw chicken samples from Lahore Another research from Lahore revealed E. coli frequency up to 78% while a Karachi investigation found 9 out of 10 samples positive for both coliforms and faecal coliforms[11,12]. Butchers' inadequate hygiene during slaughtering and meat processing was also noted in these investigations. E. coli is common in raw chicken meat in Bangladesh and Nepal[13]. The aforementioned research show that E. coli is the best indication of food and water faeces. It may be spread by contaminated hands or water-intensive slaughter methods. Proteus spp. was 15.8% in this research and 18.4% in Nepal[14]. In Peshawar Pakistan found 11 Proteus spp. out of 231 isolates, whereas Saudi Arabia found 6 from 75 meat product samples demonstrating stronger hygienic practices[15].This research detected 6.3% Enterobacter spp., comparable to another that found 6.9% Present investigation found 6 (6.3%) Citrobacter spp., which matched 3.4% but greater numbers were recorded in many studies from across the globe, making it a major meat rotting bacterium[16].This investigation found lower counts of S. aureus than a nearby study that found 55% of retail chicken samples contaminated 62% and 66.6% This investigation failed to isolate Salmonella and Campylobacter[17]. The explanation may be overuse of antibiotics for broiler chicken development and illness treatment. Rabia and Sidrah found antibacterial activity in 73.3% of 90 chicken meat samples from various parts of Lahore at University of Health Sciences. This shows that substantial doses of chicken flesh antimicrobials inhibited most microorganisms Pakistan, Iran, and Egypt also found various antibiotics in chicken parts[18]. Table 3 shows that the prevalent pathogen, E. coli, was 100% ampicillin-resistant and 98.5% ciprofloxacin-resistant. Ciprofloxacin is a powerful antibiotic for gastrointestinal and urinary tract infections. Health officials worry that multi-drug-resistant urinary strains of E. coli identified from Lahore may originate from this antibiotic[19]. Results were similar shows tetracycline, sulfonamide, and quinolone resistance. A recent research in Turkey found 97% penicillin-resistant E. coli and 94.29 % multidrug-resistant[20].The sensitivity pattern of Proteus spp. in our investigation matched Compared to this investigation, identified several Citrobacter spp. The extensive use of common antimicrobials as prophylactics and growth promoters in veterinary medicine explains the high frequency of antimicrobial resistance seen in this research[21]. Increased animal exposure to antimicrobials

causes germs to become resistant, which is then passed on to people via a flawed food chain. The rise of resistant microorganisms increases the burden of human illnesses that do not respond to most medications[22].

CONCLUSION

Based on the results of this study, majority of chicken meat samples were microbiologically fit for human use. This was further assured by the absence of Salmonella and Campylobacter in these samples. The presence of various Enterobacteriaceae in raw meat can be the potential source of infection through consumption of undercooked meat. There is a chance of cross-contamination of other food products also. One of the critical findings is presence of multi drug resistant strains of these organisms. This poses an imminent threat to the health of the community making it even more difficult to treat the gastrointestinal infections produced by them. It is thus, the need of hour, to not only educate meat handlers at basic level but also emphasize the importance of implying stern laws for the safety of food.

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Author's Contribution:

Concept & Design of Study:	Nida Javed
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Revisiting Critically:	Nida Javed, Saima Pervaiz
Final Approval of version:	Nida Javed

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