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Original Article

Microbiological Analysis of Meat and Their Control

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ABSTRACT

The current study was performed in order to check the microbial load of beef and chicken collected from the retail shops of an open area and market where that is available in preserved form. **Objective:** To compare the microbial load between the meat of an open market area and commercially preserved meat (chicken and beef). The antibiotic resistance profile of isolated pathogens was also checked. **Methods:** Ten samples of each meat specie (beef and chicken) were analyzed for the presence of different pathogens like *Salmonella*, *Shigella*, and pathogenic *Escherichia coli* mainly. The microbial load was approximately the same in beef as well as chicken. The obtained isolates were then subjected to antimicrobial resistance testing by disc diffusion method. Resistance to chloramphenicol, ampicillin and trimethoprim was determined most frequently. **Results:** In contrast, the bacterial isolates from beef samples were rarely tested resistant or simply non-resistant as compared to that off chicken samples. The bacterial isolates from chicken samples were tested highly resistant against chloramphenicol, trimethoprim and ampicillin. **Conclusion:** The significant importance of our findings is resistant rate against bacterial pathogens in chicken seems to be much higher than in beef samples found in variety of environment (different localities).

INTRODUCTION

Livestock is a vital com a central component in the agricultural sector of Pakistan and employment to about 8 million people. Meat and meat products are vital in achieving dietary needs since they are a primary source of protein and include key vitamins and minerals. Due to rising population, economic levels, and dietary options, consumer demand for nutritious, sanitary, and safe meat and meat products is expanding globally. Food preferences are largely impacted by geography, religion, and socioeconomic status. Religion, on the other hand, is one of the key determinants of eating preferences. [1]. Human illnesses with foodborne Salmonella and pathogenic Escherichia coli are suspected to be spread through contaminated cow meat. The prevalence of Salmonella and pathogenic E. coli in bovine meat and products varies substantially from one product to the next, as well as between countries. In bovine carcasses, the frequency is reduced when proper cleanliness and slaughter circumstances are maintained [2]. The availability of healthy and safe food is a basic requirement for human health. Pakistan is a tropical country with optimal circumstances for bacteria to grow and infect meat, rendering it unsafe for human consumption. The majority of the populace consumes meat that has been butchered and sold in small local markets, where hygiene is always an issue [3,4]. Bacteria can infect meat through blood, gastrointestinal contents, feet, hide or skin, water, blades, and tools used in slaughterhouse trucks and people, either directly or indirectly. The most frequent bacteria found in meat include E. coli, Staphylococcus aureus, Listeria monocytogenes, Salmonella, Aeromonas spp., Aerobacter spp., Bacillus cereus, Campylobacter spp., Clostridium botulinum, and Helicobacter species. Before it reaches consumers, veterinary inspectors should reject diseased animal meat including Bacillus anthracis, Mycobacterium tuberculosis, and Brucella abortus [5,6]. The bulk of these organisms have been shown to cause significant food-borne infections and food deterioration, posing a substantial danger to human health and the country's economy. Insanitary slaughterhouses, butcher shops, meat handling, ambient conditions, and inappropriate meat packing and selling all contribute to the infection source. Contaminated raw beef is one of the most prevalent causes of foodborne illness [7]. To control food-borne illnesses and keep the microbial load of raw meat in check, food safety requirements should be strictly followed in accordance with HACCP (hazard analysis critical control point). However, in developing countries like

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Pakistan, the abattoir environment, its sanitary level, and transportation and storage conditions not only contaminate but also enhance the growth of different types of spoilage as well as pathogenic bacteria in meat [8]. The purpose of this study was to investigate microbial contamination in raw meat (beef/bovine meat) in the context of local slaughter and market settings. [9]. Poultry meat is noted for having a high nutritional density despite its low-calorie level. Poultry meat is a good source of high-quality protein, much like beef and other meats (20-22 percent) [10]. Bacterial deterioration of meat is influenced by the number of microorganisms present at the start, the time/temperature combination of storage conditions, and the meat's physicochemical qualities [11]. Furthermore, bacteria's adhesion characteristics and the production of biofilms on surfaces make cross-contamination easier [13]. Staphylococcus, Escherichia coli, and Bacillus cereus are all sources of Staphylococcus, Escherichia coli, and Bacillus cereus in pre-slaughter conditions such as feeding and housing, which include spreadable contaminations from skin and feces, contents of the digestive system, and contaminated water [12]. Various slaughterhouse techniques, like evisceration, can infect carcasses and equipment with gut bacteria [14]. Enterobacter, Citrobacter, and Klebsiella are the most often found fecal coliforms in slaughterhouses [12,15, 16]. Antibiotic-resistant enterococci have been routinely isolated from corpses of cattle, poultry, and pigs, as well as fresh meat [17]. Pseudomonas aeruginosa is the most common spoilage bacteria in meat, owing to their metabolic versatility and ability to produce extracellular proteases and lipases [18,11]. Salmonella isolates are more common in raw beef products than E. coli O157, owing to the bacteria's superior capacity to survive outside of the animal's stomach [19]. Using culturing and PCR-based methodologies, the prevalence of E. coli and Salmonella in beef and sheepmeat varies significantly from survey to survey, ranging from 11.9–50 percent to 7.1–33 percent for each bacterium, respectively [20-22]. Human intestinal infections are linked to a wide variety of verotoxigenic Escherichia coli serotypes [23]. Some of these serotypes are known to be major foodborne pathogens that can cause bloody diarrhea and hemolytic uremic syndrome [24]. Chilled raw beef is a primary source of pathogenic E. coli, and it is thought that such germs are transmitted to meat during slaughter and processing through cattle excrement. Cattle and their surroundings are among the most common sources of pathogenic E. coli, and they may be the source of meat and meat products contamination. Cattle have also been linked to the transfer of E. coli to humans in an indirect manner [25]. As a result, in this era of rising consumption and production, guaranteeing the microbiological safety of chicken meat products is critical. Pathogenic species such as Salmonella and Campylobacter, the two primary pathogens responsible for human gastroenteritis due to chicken meat intake, may be found in the bacterial populations present in poultry meat [26]. Bacterial contamination can come from a variety of sources, including equipment surfaces, water, and animal microbiota. Broiler meat can be contaminated by bacteria from the air and the environment [27]. Because the skin of chicken carcasses and cuts comes into direct touch with air and equipment surfaces, it is quickly contaminated. Bacteria are found on the surface of fresh meat rather than in the flesh [28]. Bacteria can move into the muscles of processed foods, such as those that have been marinated.

METHODS

Different glassware, apparatus and equipment utilized during the experiment including petri dishes, conical flasks, test tubes, beaker, micro pipette tips were sterilized using autoclave. Autoclave, weighing balance, pH meter, incubators along were also used during the current research work. Total 20 Meat Samples (10 from Chicken and 10 from Beef) were collected randomly from meat retail shops located in three areas of Lahore i.e. Bhatta choke, Walton Main Saddar Market, Lahore and also from the malls (hyper star, mall of Lahore) of Lahore where they were available in processed form. The meat samples collected from retail shops were freshly used, while the other one in packed form were used frozen form (Not fresh enough). To minimize microbial alterations owing to ambient temperatures and post-slaughter timings, samples were collected within 12 hours of slaughter and early in the morning. For the analysis of microbial load in collected samples i.e. the minced meat (Chicken and Beef) samples were serially diluted and tenfold serial dilutions were prepared by weighing one-gram meat (Chicken and Beef) separately and then both samples were added in 10 ml sterile distilled water in test tubes. The suspensions obtained were transferred into the next two test tubes containing 9ml sterile distilled water until 10^{-10} dilutions were prepared. All the dilutions were plated on L agar and then incubated at 37 ℃ for approximately 24-48 hours. After 24 hours the microbial colonies were counted on each plates and microbial load was calculated by following formula. **CFU/ml in original sample = No. of colonies counted/ (Dilution Factor) (Vol plated, in ml.** CFU is a unit used to estimate the number of viable bacteria cells in the samples. For the isolation of pathogens, firstly the dilutions were made as one-gram minced meat (beef and chicken) sample was added in test tube having 10ml distilled water. With the help of micropipette 100 µl solution was taken from the test tube and were plated on L agar, SS agar and M agar media and then incubated at 37℃ for approximately 24-48 hours. Every single pathogen obtained has its own color, size, shape, margin, and elevation. Individually, the colonies were counted from each of the plate and the above parameters were then noted in

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each of the pathogenic colonies. The bacterial colonies obtained were inspected and purified through streaking technique. The LOUIS test (Table 1) is a quick, cost-effective screening test that avoids unwanted identifications by selecting isolates from traditional agars for further testing or eliminating isolates that are not suitable for further testing using a few numbers of biochemical characteristics. It had a sensitivity of 100 percent and a specificity of 94 percent as a screening protocol for Salmonella and Shigella using rapid enzyme tests, and it achieved presumptive reporting and confirmation of Salmonella and Shigella three hours after colony isolation with time and money savings compared to commercial identification systems. Firstly, the colonies were picked up from the agar plates then were added in a test tube having distilled water then a tablet of ONPG (o-nitrophenyl-beta-D-galactopyranoside) was added in a test tube. Indication of colorless or light yellowish colonies ensured the conformation of presence of SS colonies in the test tube. Later on, to confirm further Urease (URE) tablet was added in the test tube having bacterial colonies. It also confirmed the presence of SS colonies by giving light yellowish color.

The louis test		
L	=	Lysine Decarboxylase (LDC)
O	=	ONPG (o-nitrophenyl-beta-D-galactopyranoside)
U	=	Urease (URE)
I	=	Indole (IND)
S	=	Screen Test

Table 1: LOUIS test

The spot indole test is used to characterize colonies and quickly identify E. coli infections. The morphologic criteria/spot-indole technique correctly identified pathogens on M agar as *E. coli*. The spot-indole test was performed on these isolates using either a 1% or 5% p-dimethyl amino benzaldehyde or a 1% p-dimethyl amino cinnamaldehyde reagent. To begin, a piece of filter paper was soaked with 1 percent p-dimethyl amino cinnamaldehyde reagent. Then, the bacterial colonies were picked up from agar surface with the help of wooden stick and bacteriological loop and were rubbed on the filter paper. Appearance of pink spot on the filter paper indicated the presence of *E. Coli* pathogens. Antibiotic resistance profile of isolated pathogens was checked. For this purpose, the suspensions of pathogens were prepared from obtained colonies of pathogens that were picked up by an inoculating loop in a laminar flow cabinet and then were added in test tubes having liquid media broth (Table 2). Inoculated test tubes and were incubated for about 24 hours approximately. After obtaining bacterial growth in test tubes, bacterial culture was picked with the help of micro pipette and poured on M agar And SS agar plates separately after pouring, swabbing is done with the help of sterile cotton swabs. Then commercially prepared antibiotic discs were placed on these plates having bacterial inoculation. Inoculated plates were incubated at 37°C to check the resistance of pathogens against antibiotics.

Pathogenic strains	**Antibiotic concentration (µg/ml)**														
	Penicillin			**chloramphenicol**			**trimethoprim**			**Ampicillin**			**Erythromycin**		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30

Table 2: Antibiotic and their concentration used for pathogenic resistance.

RESULTS

Isolation of bacterial pathogens is as follows. In total 20 samples (10 of each meat type) were taken for isolation of bacterial strains or pathogens. The bacterial colonies or strains were obtained after 24 to 36 hours of incubation on M agar and SS agar media. Approximately same number of colonies were obtained on both beef and chicken samples. M agar is a selective media for the growth of gram-negative *E. coli* bacteria and it produced bright pink colonies of *E. coli* mainly. Whereas SS agar is selective as well as differential media for the growth of *Salmonella* and *Shigella* bacteria mainly produced colorless colonies of both *Salmonella* as well as *Shigella*. It also gives circular, smooth, convex and transparent colonies of *Shigella*. Louis test confirmed the presence of *Salmonella* and *Shigella* by producing black or transparent or sometimes light yellowish color. Whereas spot-indole test confirmed the presence of *E. coli* by producing bright pink color. The obtained bacterial

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colonies were further streaked for purification. Bacterial colonies observed on these agar media were also counted. From the obtained results of bacterial isolation from beef and chicken, all the samples showed the presence of bacterial pathogens.

Beef				**Chicken**	
Samples	**Agar media**	**Open**	**preserved**	**Open**	**preserved**
1	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
2	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
3	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
4	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
5	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
6	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
7	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
8	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
9	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve
10	SS agar	+ve	+ve	+ve	+ve
	M agar	+ve	+ve	+ve	+ve

Table 3: Presence/absence of pathogens in beef and chicken samples collected open and preserved areas

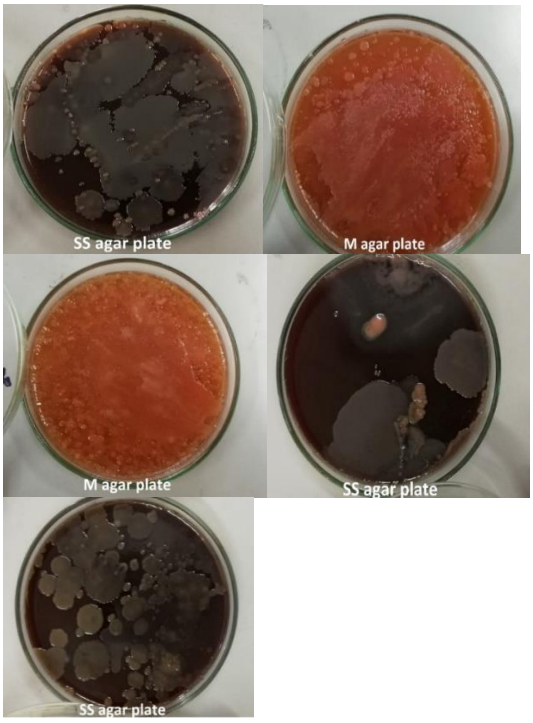

Figure 1: Microbial flora observed in the chicken samples collected from different shops.

Antibiotic resistance profile of pathogens isolated from beef and chicken samples. Chlormphenicol, erythromycin, penicillin, trimethoprim and streptomycin discs were used for the control of bacterial colonies or pathogens on the agar

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plates (L agar, M agar, SS agar). Unfortunately, bacterial colonies on beef were non-resistant against these antibiotic discs. Whereas some of the chicken samples showed resistance against the applied antibiotic discs. Antibiotic discs removed the matte of bacterial colonies around them (Table 3).

Pathogenic strains	Antibiotic concentration (µg/ml)														
	Penicillin			Chloramphenicol			Trimethoprim			Ampicillin			Erythromycin		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
1.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 4: Concentration of antibiotic discs used against microbial flora of beef

E. coli, Salmonella, Shigella

In table 4, Penicillin, Chloramphenicol, Trimethoprim, Ampicillin and Erythromycin were used with different concentrations for checking the bacterial resistance from collected beef samples. Firstly, penicillin with a concentration of 10 µg, 20 µg, and 30 µg showed no resistance against the obtained bacterial pathogens. Afterwards chloramphenicol, trimethoprim, ampicillin and erythromycin were also applied with a concentration of 10µg, 20µg, and 30µg on the bacterial pathogens on agar plates. They all showed no resistance against the observed bacterial pathogens.

Pathogenic strains	Antibiotic concentration (µg/ml)														
	Penicillin			chloramphenicol			trimethoprim			Ampicillin			Erythromycin		
	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
1.	-ve	-ve	-ve	-ve	-ve	+ve	+ ve	_	_	+ve	_	_	-ve	-ve	-ve
2.	-ve	-ve	-ve	-ve	-ve	+ve	+ ve	_	_	+ve	_	_	-ve	-ve	-ve
3.	-ve	-ve	-ve	-ve	-ve	+ve	+ ve	_	_	+ve	_	_	-ve	-ve	-ve

Table 5: Concentration of antibiotic discs used against microbial flora of chicken

E. coli, Salmonella , Shigella

In table 5 Penicillin, Chloramphenicol, Trimethoprim, Ampicillin and Erythromycin were used with different concentrations for checking the bacterial resistance from chicken samples. Penicillin and erythromycin with a concentration of 10µg, 20µg and 30µg showed no susceptibility against the observed bacterial pathogens on agar plates. Later on, chloramphenicol with a concentration of 10µg, and 20µg also showed no susceptibility against the bacterial pathogens, but it's concentration of 30µg finally removed the bacterial matte from the agar plates, hence showing the susceptibility. Afterwards trimethoprim and ampicillin quickly showed the resistance at just 10µg.

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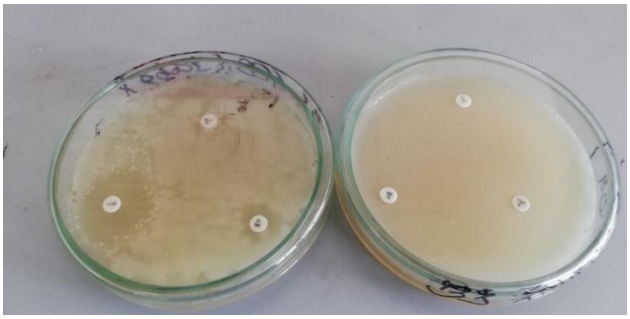

Figure 2: Antibiotic susceptibility testing by disc diffusion method on L agar media in chicken sample.

Figure 3: Antibiotic susceptibility testing by disc diffusion method on M agar media and SS agar media in chicken sample

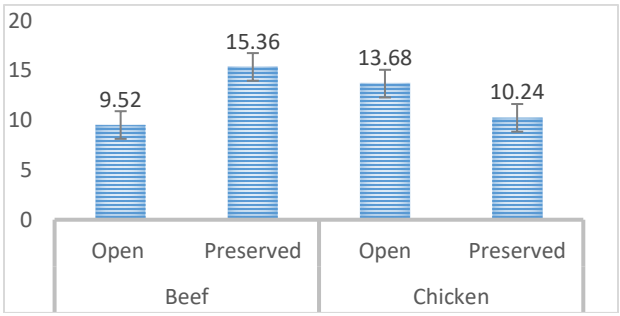

Figure 4: Comparison of Microbial load in beef and chicken samples collected in open and preserved form.

DISCUSSION

In the present study, in comparison between the samples of retail shops and that of the processed meat from the malls, it was observed that the microbial load was much greater in the meat of retail shops both in beef and chicken. While M agar medium was specifically for pathogenic *E. Coli* and SS agar medium for *Salmonella* and *Shigella* as pathogen. The isolated bacterial strains were then purified by streak plate method. Presence of bacterial pathogens observed both in beef and chicken were almost same. A study also undergone bacterial analysis of meat samples and followed two major assessments: firstly, the total viable count (TVC) and total *E. coli count* mainly on the nutrient agar, plate count agar, MacConkey's agar, blood agar, salmonella-shigella agar. He concluded that the total viable count (TVC) and *E. coli* count was great on nutrient agar, MacConkey's agar and SS agar mainly in both beef and chicken samples.

Comparing the microbial load of beef and chicken, the samples taken from retail shops and the samples in preserved form almost showed the same ratio of microbial load in both beef and chicken. But the microbial load in beef samples taken from retail shops showed the presence of 9.52% bacterial pathogens, while the chicken samples showed the presence of almost 13.68% bacterial pathogens and the microbial load in preserved samples of beef was 15.36%, while that of chicken was 10.24%. Hence the graph concluded that the chicken and beef samples showed almost same number of bacterial pathogens collectively.

Pathogens isolated from chicken were resistant to chloramphenicol, trimethoprim and ampicillin whereas the bacterial isolates from beef samples were rarely tested resistant or simply were non-resistant [28]. have also done the antibiotic susceptibility testing by disc diffusion method. He also used amoxicillin, ampicillin, ciprofloxacin, penicillin and

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tetracycline as an antibiotic and concluded that these antibiotics showed preferable resistance against beef and chicken samples.

CONCLUSIONS

Concluding the research, as the bacteria, viruses and parasites are the sources of many food borne illnesses or diseases usually due to improper handling of food or use of unsterilized equipment's during cutting and processing of meat. So, the current study recommends that the slaughter houses should be cleaned properly and the equipment's or tools during cutting or processing of meat must be sterilized before use. Most importantly it recommends the use of healthy diet for animals in order to avoid different diseases.

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