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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *SALMONELLA*, *E. COLI*, AND *S. AUREUS* FROM RAW MEAT OF CHICKEN AND MUTTON IN BAHRAIN SWAT

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ABSTRACT

Background: *There has been an increase interest in consumption of meat in many countries in recent years. It might, however, serve as a route for foodborne antibiotics resistant bacteria. Mutton and chicken are the most popular foods in Pakistan.*

Objective: *The purpose of this study was to ascertain the antibiotic resistance and prevalence of foodborne pathogens, including Salmonella, E.coli, and Staphylococcus aureus from raw meat of chicken and mutton.*

Materials and method: *The current study was carried out at the department of Microbiology Government college Madyan swat from august 2023 to May 2024. A total of 52 samples of raw meat (26 Mutton and 26 Chicken) were collected from different regions of tehsil Bahrain for the isolation and antibigram of Salmonella, E. coli, and S. aureus. These samples were inoculated on selective media and incubated for 24 hours for bacterial growth. Each bacteria was identified through gram staining and biochemical tests. For the isolates, antibiotic sensitivity testing using the Kirby Bauer's disc diffusion method was carried out using commercially available antibiotics on Muller-Hinton agar discs (MHA).*

Results: *A total of 52 raw meat samples (26 from chicken and 26 from mutton) were examined in this study. Out of which 46 samples (88.6%) (23 chicken and 23 Mutton) showed bacterial growth which were further included in this study while culture negative were excluded. The most common bacteria isolated were from raw meat of chicken was E. coli (34.7%) followed by Salmonella typhi (21.73%) and Staphylococcus aureus (8.6%) respectively. Similarly raw meat of mutton also showed highest growth of E.coli (30.4%), Salmonella (26.0%) and S.aureus (15.2%). The antimicrobial susceptibility profiles of the E.coli showed that all isolates were 100% sensitive to nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin but were resistant to tetracycline (29.4%), streptomycin (26.4%) and cefoxitin (11.5%) correspondingly. Similarly salmonella species were 100% sensitive to Nalidixic acid and Nitrofurantoin and resistant to cefoxitin, (54.5%) tetracycline (18.1%), and gentamycin (9%), respectively. The antibiotic susceptibility profiles of the isolates showed that the isolates were (69.2%) and (7.6%) resistant to Penicillin G and Erythromycin, respectively. On the other hand, all isolates were 100% sensitive to cefoxitin, nitrofurantoin, and gentamicin.*

Conclusion: *It is possible for consumers to get different infections from meat. E. coli, Salmonella, and S. aureus were identified in mutton and raw chicken meat samples, suggesting a significant risk of food safety issues. The results of the study demonstrated that pathogenic bacteria resistant to antibiotics may be present in raw chicken meat and mutton, presenting a significant risk to public health.*

Keywords: *Isolation; Identification; Antimicrobial susceptibility pattern; Salmonella, E. coli; S. aureus; Chicken; Mutton.*

INTRODUCTION

Foodborne infections and diseases are significant worldwide problems that carry substantial risks to people's health and well-being. Over two hundred and fifty diseases related to food have been identified by researchers.(1) Foodborne diseases has a significant influence on a country's social and economic output in addition to its effects on an individual's physical health and well-being.(2) The World Health Organization estimates that foodborne diseases cause 600 million cases, more than 420,000 deaths, as well as 33 million DALYs (disability-adjusted life years) every year.(3) The main cause of the rising incidence of food-borne illnesses, is the general lack of hygienic practices among individuals.Improper personal hygiene among food preparation staff and improper meat handling procedures at slaughterhouses have the potential to spread microbiological pathogens that cause foodborne diseases.[4] The main means by which that humans become infected is via eating contaminated food, especially raw or undercooked meat, mostly mutton and beef. (5) Because meat and meat products are rich in essential amino acids, fat, protein, minerals, vitamins, and other nutrients, they are very nutrient-dense and attractive to humans.(6) Meat is an excellent source of vitamins, minerals, essential fatty acids, and protein. However, because it may foster the perfect setting for the growth of many microbes, it is extremely perishable.(7) When meat comes into touch with bacteria during the processing of animal carcasses at the slaughterhouse, it can become contaminated. These pollutants might be internal, like those found in the gastrointestinal tract, or external, like those found on the skin or in the environment.(8) Salmonella species, Staphylococcus aureus, Escherichia coli, Campylobacter jejuni, Listeria monocytogenes, and Clostridium perfringes are the main foodborne bacterial pathogens that are frequently detected in meat.[9]. Consumption of chicken and dairy products has led to the development and spread of antibiotic-resistant food-borne infections in humans, which can be attributed to the increasing use of antibiotics in agriculture.(10). Therefor the current study was conducted to ascertain the antibiotic resistance and prevalence of foodborne pathogens, including *Salmonella*, *E. coli,* and *Staphylococcus aureus* from raw meat of chicken and mutton.

Materials and method

Collection of samples

The current study was carried out at the department of Microbiology Government college Madyan swat from august 2023 to May 2024. A Total of 52 samples of raw meat (26 Mutton and 26 Chicken) were collected from different regions of tehsil Bahrain for the isolation and antibigram of *Salmonella*, *E. coli*, and *S. aureus*. Meat samples (weight 100 gram) were collected in dry, and sterilized polythene bags and transported to microbiology lab within hour for the microbiological analysis or kept under refrigeration at 4°C until further investigation was done, but no later than a full 96 hours following the date of purchase. [11]For the culturing of *E.coli* the sample rinsate was inoculated on MacConkey Broth and incubated for 24 hours after that it was then plated onto Eosin Methylene Blue agar. The sample rinsate was plated into double-strength lactose broth at 37°C for 24 hours in order to isolate Salmonella spp and then inoculated on Salmonella and Shigella agar. The rinsate was inoculated into nutrient broth at 37°C for 24 hours, after which it was plated onto Mannitol Salt agar to identify Staphylococcus aureus and other non-fastidious bacteria. [12] The initial morphological examination of the colonies in the plate (macroscopically) for colonial appearance, size, elevation, form, edge, consistency, color, odour, opacity, hemolysis, and pigmentation was used to characterize and identify the colony isolates. The results were recorded. The bacteria were first identified using Gram's staining from the colonies.[13]

Antibiogram of bacteria isolated from raw meat

For the isolates, antibiotic sensitivity testing using the Kirby Bauer's disc diffusion method was carried out using commercially available antibiotics on Muller-Hinton agar discs (MHA). The isolates' standard suspensions were brought to 0.5 McFarland Standard. Following standardization, a sterile cotton swab was dipped into bacterial suspension and a lawn culture was carried out on the MHA petri-dish. The surface of inoculated plates had been covered with commercially available antibiotic discs. The plates were incubated for 16–18 hours at 37^0 C. In accordance with NCCLS standards, the antibiotics were selected. Following incubation, each antimicrobial agent's zone diameter was measured and compared to the NCCLS chart. As consequently, the zone of inhibition was categorized as resistant (R), intermediate (I), or sensitive (S) [14].

Results

For the isolation and antibiotic susceptibility pattern of *Salmonella*, *E. coli*, and *S. aureus* from raw meat of Chicken and mutton a total of 52 raw meat samples (26 from chicken and 26 from mutton) were examined in this study. Out of which 46 samples (88.6%) (23 chicken and 23 Mutton) showed bacterial growth which were further included in this study while culture negative were excluded (**figure 1).** The most predominate bacteria isolated were from raw meat of chicken was *E. coli* 18(34.7%) followed by *Salmonella typhi* 10(21.73%) and *Staphylococcus aureus* 4(8.6%) respectively. Similarly raw meat of mutton also showed highest growth of *E.coli* 16(30.4%), *Salmonella* 12(26.0%) and *S.aureus* 7(15.2%) The chicken meat was most contaminated as compared to mutton as shown in table **1.**

Antimicrobial susceptibility profiles of isolates

Antibiogram of *E.coli*

A total of 34 isolates of *E.coli* were selected and tested against eight frequently used antibiotics. The antimicrobial susceptibility profiles of the isolates showed that these were resistant to tetracycline (29.4%), streptomycin (26.4%) and cefoxitin (11.5%) correspondingly. On the other hand, all isolates were 100% sensitive to nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin as represented in **table 3**.

Antibiotic susceptibility pattern of *Salmonella typi*

All the isolates of Salmonella were tested against eight commonly used antimicrobials. The antimicrobial susceptibility profiles of the isolates showed that the isolates were resistant to cefoxitin, (54.5%) tetracycline (18.1%), and gentamycin (9%), respectively. On the other hand, all isolates were 100% sensitive to nalidixic acid and Nitrofurantoin as presented in **table 2.**

Antibiotic susceptibility pattern of *S.aureus*

All the isolates of S.aureus were tested against 9 commonly used antimicrobials. The antibiotic susceptibility profiles of the isolates showed that the isolates were (69.2%) and (7.6%) resistant to Penicillin G and Erythromycin, respectively. On the other hand, all isolates were 100% sensitive to cefoxitin, nitrofurantoin, and gentamicin as described in **table 4**.

Table 1. Frequency of pathogenic bacteria isolated from raw meat of Chicken and Mutton

Sample	Isolated bacteria	Number	Percentage
Raw meat of chicken	***Escherichia coli***	**18**	**34.7%**
	Salmonella typhi	**10**	**21.73%**
	Staphylococcus aureus	**4**	**8.6%**
Mutton	***Escherichia coli***	**16**	**30.4%**
	Salmonella typhi	**12**	**26.0%**
	Staphylococcus aureus	**7**	**15.2%**

Table 2. Antimicrobial Sensitivity test results of Salmonella isolates from Mutton and chicken raw meat

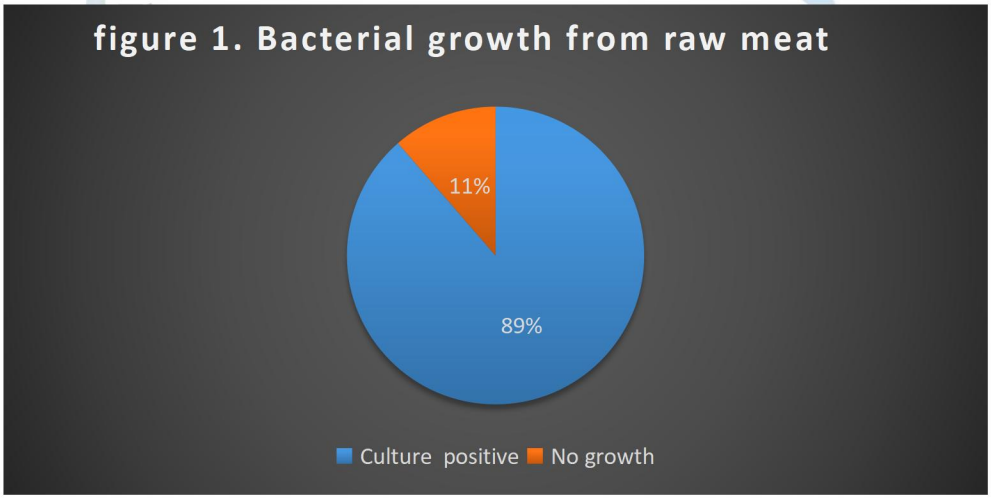
Antibiotics	Disc concentration (µg)	No of isolates tested	Resistant	Intermediate	Susceptible
Nitrofurantoin	250	22	0	0	18(81.8%)
Tetracycline	25	22	4(18.1%)	0	18(81.8%)
Ciprofloxacin	5	22	4(18.1%)	6(27.2%)	12(54.5%)
Ceftriaxone	5	22	0	4(18.1%)	18(81.8%)
Cefoxitin	25	22	12(54.5%)	0	8(36.36%)
Streptomycin	10	22	0	12(54.5%)	10(45.4%)
Nalidixic acid	25	22	0	0	22(100%)
Gentamycin	10	22	2(9%)	12(54%)	8 (36.3%)

Table . 3 Antimicrobial Sensitivity test results of *E.coli* isolates from Mutton and chicken raw meat

Antibiotics	Disc concentration (µg)	No of isolates tested	Resistant	Intermediate	Susceptible
Nitrofurantoin	250	34	0	0	34 (100%)
Tetracycline	25	34	10(29.4%)	4(11.7%	20(58.8%)
Ciprofloxacin	5	34	0	0	34(100%)
Ceftriaxone	5	34	0	0	34(100%)
Cefoxitin	25	34	4(11.5%)	0	30(88.23%)
Streptomycin	10	34	9(26.4%)	5(14.7.5%)	20(58.4%)
Nalidixic acid	25	34	0	0	34(100%)
Gentamycin	10	34	0	15(44.1%)	19(55.8%)

Table. 4 Antimicrobial Sensitivity test results of *S.aureus* isolates from Mutton and chicken raw meat

Antibiotics	Disc concentration (µg)	No of isolates tested	Resistant	Intermediate	Susceptible
Penicillin G	10	13	9(69.2%)	0	4(30.7%)
Azithromycin	25	13	0	1(7.6%)	12(92.3%)
Erythromycin	15	13	1(7.6%)	2(15.3%)	10(76.9%)
Nitrofurantoin	250	13	0	0	13 (100%)
Tetracycline	25	13	0	9(69.2%)	3(58.8%)
Ciprofloxacin	5	13	0	3(23.0%)	10(100%)
Cefoxitin	5	13	0	0	13(100%)
Streptomycin	25	13	0	1(7.6%)	11(88.23%)
Gentamycin	10	13	0	0(14.7.5%)	13(100 %)



Discussion

Meat contaminated by bacteria is common because they are found both in animals and their environments. It has a direct impact on spoiling and shelf life of raw meat. So evaluating the initial amount of bacteria in meat is crucial.[15]

In this study we examined the raw meat of chicken and mutton for the isolation and antibiogram of *Salmonella*, *E. coli*, and *S. aureus.* The most prevalent bacteria isolated in the current study from raw meat of chicken and mutton was *E. coli* (65.1%).Our study findings are similar to a research conducted in

Switzerland by Roger et al.in 2004 in which they isolated *E.coli* the most predominant bacteria from raw meat.[16] *E. coli* is considered an imminent risk to meat sector and general public health. Escherichia coli is thought to indicate poor hygiene and sanitary standards during slaughtering and subsequent handling in addition to fecal contamination. Additionally, this study indicates that the biggest barrier to meat storage in the study region is *E. coli*. The finding of our study however, is more than that of Bedasa et al.[17] who noticed a 3.5% prevalence of *E.coli* in meat originating from cattle. The differences in results between this study and previous studies might be attributed to disparities in management practices, hygienic standards, and environmental factors. Salmonella species continue to be one of the most significant food-borne diseases in the world. Food-borne salmonellosis has increased significantly in recent years, with outbreaks reported in a number of nations, including Spain, Italy, England, and America. A variety of foods, including chicken, eggs, meat, fish, dairy products, and chocolate, have been associated with outbreaks. 47% of the isolates of Salmonella were found in our research. These findings are not similar compared to those of Suad and Wisam [18] who recovered Salmonella (58%), from beef meat. The 3rd most common bacteria isolated in our study was S.aureus (23.8%).These findings are not similar to Bantawa et al.[19] they isolated 68% which is quit higher than the present study. There might be a reason for the discrepancies in rates of prevalence between this study and others, such as improper handling and inadequate cleaning practices in meat markets. The investigation found that meat retailers were unaware of basic standards and regulations related to meat. The improper use of antibiotics in veterinary and human medicine has led to the emergence and spread of bacteria resistant to antibiotics.[20] The antimicrobial susceptibility profiles of the Salmonella showed that the isolates were resistant to cefoxitin, (54.5%) tetracycline (18.1%), and gentamycin (9%), respectively. These results are not similar with the study conducted by Addis, et al in which they reported high resistance gentamycin (25%) tetracycline (30%).[21] According to a research conducted in Alexandria, Egypt, by Mohamed, et al. [22], tetracycline was effective against 85.7% of the Salmonella species that were isolated from dairy cattle. it was evaluated from the current study that all the isolates of *E.coli* were 100% sensitive to nitrofurantoin, ceftriaxone, nalidixic acid, and ciprofloxacin. These results are similar with the study conducted by Salehi and Bonab [23] in Iran in which *E.coli* found 100% sensitive to to these antibiotics. The antibiotic susceptibility profiles of the *S.aureus* showed that the isolates were (69.2%) and (7.6%) resistant to Penicillin G and Erythromycin, respectively. On the other hand, all isolates were 100% sensitive to cefoxitin, nitrofurantoin, and gentamicin. Some researchers believe that prolonged, improper, and careless use of penicillin G-resistant isolates may be contributing to their rising occurrence.[24] The present study assessed that S. aureus exhibited resistance to penicillin G and erythromycin, with percentages of 69.2% and 7.6%, respectively. These results are in contrast to those of Sori et al. (87.2%) [25] in Ethiopia and Landin (80%) [26] in Sweden, but they are in strong agreement with Gooraninejad et al. (68%) in Iran [27].

Conclusion

It is possible for consumers to get different infections from meat. *E. coli*, Salmonella, and *S. aureus* were identified in mutton and raw chicken meat samples from Bahrain marketplaces, suggesting a significant risk of food safety issues. Therefore, regular microbiological investigation should be implemented in slaughterhouses, markets, and other meat-rendering facilities. Furthermore, regardless of the source of the meat in the market, consumers must handle and cook meat properly to prevent foodborne disease. The results of the study demonstrated that pathogenic bacteria resistant to antibiotics may be present in raw chicken meat and mutton, presenting a significant risk to public health.

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