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Research Article

Evaluation of Bacterial Contamination From Raw and Cooked Fish, Mutton and Beef Sold by Local Vendors in Hyderabad, Pakistan

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Abstract | Foodborne pathogens are spreading to humans through contamination of raw and cooked meat because of its inappropriate processing, handling and cooking. Therefore this research was performed for the evaluation of bacterial contamination from raw and cooked fish, mutton and beef sold by retailors in district Hyderabad. During present study, 150 total meat samples, 100 from raw meat (beef=40, mutton=40, fish=20) and 50 from cooked meat (beef=20, mutton=20, fish=10) were randomly collected from district Hyderabad and cultured on different media for isolation of bacterial species. The isolated species were identified by different biochemical tests. The results showed that the contamination of bacterial organisms in both raw and cooked meat was highest in beef followed by mutton and fish respectively ($p < 0.05$). In raw meat, bacterial species recorded were *Escherichia coli* (45%, 30% and 25%), *Salmonella enteritidis* (20%, 17.5% and 15%), *Staphylococcus aureus* (30%, 25% and 25%), *Bacillus cereus* (12.5%, 10% and 10%), *Klebsiella pneumoniae* (15%, 10% and 0%) and *Shigella dysenteriae* (10%, 12.5% and 5%) in beef, mutton and fish respectively. While from cooked beef, mutton and fish the prevalence of *E. coli* (25%, 25% and 20%), *S. aureus* (15%, 15% and 10%), *S. enteritidis* and *B. cereus* (10%, 10% and 10%) were observed. The highest ($p < 0.05$) bacterial load (g^1) was detected in raw (1.76×10^6) and cooked (6.1×10^4) beef than raw (1.55×10^6) and cooked (4.5×10^4) mutton respectively, while raw (1.25×10^6) and cooked (2.9×10^4) fish exhibited the least ($p < 0.05$) bacterial load than other raw and cooked meat (beef and mutton) respectively. Data regarding antimicrobial susceptibility exhibited that among eight antibiotics *E. coli, S. enteritidis, S. aureus* and *B. cereus* were observed sensitive to gentamycin, norfloxacin and ciprofloxacin. *K. pneumoniae* showed sensitivity against gentamycin, norfloxacin, ciprofloxacin, erythromycin, tetracycline and streptomycin; whereas *S. dysenteriae* was observed sensitive to gentamycin, norfloxacin, tetracycline and ampicillin. In conclusion, raw beef samples were found more contaminated than raw fish and mutton while cooked fish samples were observed less contaminated than cooked beef and mutton. Furthermore, all bacterial isolates (except *K. pneumoniae*) were found multidrug resistant.

Keywords | Microbial load, Raw fish, Cooked meat, Beef, Foodborne pathogens

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INTRODUCTION

Meat is a nutrient-rich food that has more bioavailability than other foodstuffs with essential quantities of proteins, vitamins and minerals (McAfee et al., 2010). Since ancient time, it has been known for its' high composition of nutrients which make it an ideal food item around the world. Meat is considered a most perishable food because its' water activity and ideal pH offers favourable environment for the growth of microbes. During storage, collection, preparation and delivery, cross contamination of meat and meat products usually occurs (Dave & Ghaly, 2011).

Due to rich and nutritious nature, meat and meat sources have become increasingly part of the daily human diet. Between bacterial species, *Yersinia enterocolitica*, *Campylobacter* spp., *Bacillus cereus*, *Salmonella* spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Listeria monocytogenes* and *Escherichia coli* are the typical pathogens related with quality of meat or meat products (Biswas et al., 2011). Food borne infections were developed in humans via different pathogens such as, *Campylobacter, E.coli, Salmonella, C. perfringens, L. monocytogenes, Helicobacter and Arcobacter* (Corry and Attaby, 2001). Oladipo and Bakole, (2013) described that compared to mutton, chicken, beef and bush meat, fish is more palatable and digestible, but can also be a cause of transmission of various types of microbes. In ruminants' meat, microorganisms spread predominantly by contaminated food and water and by faeco-oral route or improper storage and handling. Whereas, in fish, water, salt contents, temperature, distance between fishing and storage points, and handling and processing are main sources of contamination (Huss et al., 2000; WHO, 2000).

Due to quick urbanization and hasty life style, society's eating ways have changed dramatically leading to increased demand for meat products particularly ready to cook and ready to eat. A research showed that cooked samples of mutton meat were infected with *E.coli*, *Aeromonas* spp., *Salmonella*, *E.coli* 0157:H7 and *S.aureus*, whereas uncooked samples were highly contaminated with *Aeromonas* spp., *Salmonella*, *E.coli*, *S.aureus* and *E.coli* 0157:H7 (El Shrek and Ali, 2012). Likewise a study from Ethiopia reported the 1.9 to 12.1% microbial contamination (viz., bacteria, mould and yeast) in cooked fish, beef, mutton and chicken samples (Bedada et al., 2020). In an Egyptian study, all (n=83) chicken meat samples were found contaminated by colistin sulfate resistant *E.coli* (Sorour et al., 2022). It has been observed that sanitary conditions at raw and cooked food-selling points is not appropriate in district Hyderabad. Therefore, the current study was designed to evaluate the bacterial contamination of raw and cooked fish, beef and mutton sold in different regions of Hyderabad, Pakistan.

MATERIAL AND METHODS

Sample collection

A total of 150 meat samples, 100 from raw meat, [beef (n=40), mutton (n=40), and fish (n=20)] and 50 from cooked meat, [beef (n=20), mutton (n=20), and fish (n=10)] were collected hygienically from local vendors of different regions of Hyderabad district in a sterile labelled plastic bags. Cooked meat samples includes grilled, smoked, fried, curry, etc. The collected meat specimens were carried to the laboratory and kept in refrigerator at 4°C until analyzed.

Processing of meat

A 25 gram of each meat sample was homogenized in 225ml of sterile peptone water. It was incubated at 37 °C for overnight than used for bacteriological examination (Zhang et al., 2016).

Bacteriological examination

Samples (meat) were cultured on different bacteriological media (Oxoid, UK) and kept for 24 hours at 37°C to obtain the colonies of bacteria. The bacterial colonies were used to prepare slide and were stained by Grams' staining to know the characteristics of bacteria. Biochemical assessments were performed to determine the species of bacterial isolates on the basis of their biochemical characteristics (El-Bayomi et al., 2020; Musawa et al., 2021). Then antibiotic sensitivity test was performed to assess the sensitivity of pathogenic organism against eight different antimicrobials i.e., norfloxacin, tetracycline, neomycin, gentamicin, ampicillin, streptomycin, ciprofloxacin and erythromycin. The breakpoints of different antimicrobials for resistant were adopted as per the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012). Antimicrobial disks were purchased from a commercial company (Oxoid, UK). All samples were run in triplicates.

Statistical analysis

The data was entered into a computer database using the Microsoft Excel spread sheets (Microsoft Inc., USA). The variance between the occurrences of microbial contaminants in fish, mutton and beef samples was compared by Fisher's exact test at 5% probability level using JMP statistical package software (version 5.0.1.a, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Prevalence of bacteria in raw fish, mutton and beef

Foodborne diseases and infections is a significant public health problem that has a negative economic impact. It is

a leading cause of sickness and death all over the world (Adak et al., 2005). In terms of pathogens and other potential pollutants, animal products such as meats, fish and their products are commonly known as high risk commodities (Yousuf et al., 2008). In present study, prevalence of bacterial species in raw fish, mutton and beef were represented in Table-1. Results exhibited that the prevalence of *E.coli* was found statistically different ($p < 0.05$) in various types of meats. *E.coli* was observed in 45% beef, 30% mutton and 25% fish samples. *S. enteritidis* was found higher ($p < 0.05$) in beef (20%) than mutton (17.5%) and fish meat (15%). *S.aureus* was also found higher ($p < 0.05$) in beef (30%) than mutton and fish meat (25% each). *B.cereus* exhibited a slightly higher ($p > 0.05$) prevalence in beef (12.5%) than mutton and fish meat (10% each). *Klebsiella pneumoniae* showed significantly higher ($p < 0.05$) prevalence in beef (15%) and mutton (10%) as compared to fish (0%). Likewise, *Shigella dysenteriae* exhibited higher prevalence ($p < 0.05$) in beef (10%) and mutton (12.5%) than fish samples (5%). The study of Jahan et al. (2015) in Sylhet Sadar also reported some observation similar to our study, however prevalence of some microorganisms were not consistent to our study probably due to variation in hygienic conditions in various meat slaughtering or selling areas. They reported the prevalence of *E.coli* (10%), *Salmonella* spp. (13.33%), *Klebsiella* spp. (20%), *Enterobacter* spp. (6.67%) and *S.aureus* (26.6%) in raw beef. Bantawa *et al*., (2019) reported the prevalence of *S.aureus*, *E.coli*, *Salmonella* and *Shigella* as 68%, 53%, 35% and 6% respectively from raw buffalo, and chicken meat in eastern Nepal. Onyango et al. (2009) recorded 39.7% *Shigella* spp., 6.3% *S. enteritidis* and 25.4% *E.coli* from fish in Kenya.

Prevalence of bacteria in cooked fish, mutton and beef

The data regarding prevalence of bacterial species in cooked fish, mutton and beef were represented in Table-2. From the data it was clear that, the prevalence of *E.coli* in beef (25%) and mutton (25%) was higher ($p < 0.05$) than fish meat (20%). Similarly, *S.aureus* prevalence was observed higher in beef (15%) and mutton (15%) as compared to fish (10%). However, *S. enteritidis* and *B.cereus* were recorded in 10% of each cooked beef, mutton and fish meat samples. Similar investigations done by Gamal et al. (2020) who found *B.cereus*, *E.coli*, *Salmonella* and *S.aureus* in beef burger with incidence rate of 6%, 6%, 0% and 8% respectively, while that in kofta was 14%, 12%, 2% and 24% and from sausages was 12%, 10%, 2% and 16% respectively. Hassanien et al. (2018) reported the *B.cereus* in fried Tilapia, grilled Mackerel and Sardine fishes with incidence rate of 20%, 18% and 30% respectively. Mohamed, (2012) reported that the incidence of *E.coli* in grilled and fried fish were 16.7% and 6.7% while *S. aureus* prevalence rate were 20% and 13.3% respectively. `observed the incidence of *S.aureus* in meals from cafeterias and reported the prevalence rate of *S.aureus* as 16.9%, 9.7%, 6.2%, 11.3% and 7.1% from meatballs, beef, mutton, hamburger and salmon respectively.

Bacterial load in raw and cooked beef, mutton and fish

Results presented in Table-3 showed that the highest ($p < 0.05$) bacterial count was detected in raw (1.76×10^6) and cooked (6.1×10^4) beef than raw (1.55×10^6) and cooked (4.5×10^4) mutton respectively, while raw (1.25×10^6) and cooked (2.9×10^4) fish exhibited the least ($p < 0.05$) bacterial load than other raw and cooked meat (beef and mutton) respectively. All raw meat samples exhibited the higher ($p < 0.05$) bacterial load as compared to their corresponding cooked meat. Similar results were recorded by Bughti et al. (2017) who reported higher bacterial count (4.1×10^9 CFU/g) in raw beef than mutton (3.9×10^7 CFU/g) and butcher's equipment sample (3.7×10^6) respectively. Ayaz et al. (1985) reported bacterial count as 3.0×10^8 CFU/g in beef, chicken and lamb shawarma from different restaurants. Mohamed, (2012) found the bacterial count in grilled and fried fish were 22.3×10^5 and 2.1×10^5 respectively.

The results of total bacterial count of meat samples were not found within the standard requirements of the International Commission on Microbiological Specification (ICMS, 1982) ($<1.0\times10^6$ cfu/g). Likewise, the results of total bacterial load were higher than the standard range for fresh fish according to (ICMS, 1986). It is generally accepted that fish with microbial load of $>10^6$ cfu/ml is likely to be at the stage being unacceptable from the microbiological point of view and unfit for human consumption (Cheesbroughl, 2000). Whereas, the values of total bacterial count of cooked mutton were less than the critical limits (3×10^5 cfu/g) set by French regulations (DGAL, 2000). In advanced countries, regulatory bodies have set a spoilage limit (i.e., 10^6 cfu/g) for meat that must not be present for sell to consumers (Nieto et al., 2010). The level of bacterial load in meat that have observed in our study indicated that raw meat sold in our local markets with open retail outlets contains a hazardous level of viable spoilage organisms that could be potential threat to meat spoilage as well as consumer's health (Ali et al., 2010). However, cooked meat (beef, mutton and fish) were found safe for human consumption that probably due to full cooking practice (at high temperature) in our local cuisine.

Antimicrobial sensitivity

In current study, results regarding antimicrobial sensitivity presented in Table-4 showed that *E.coli* was observed sensitive to gentamycin, norfloxacin and ciprofloxacin (19, 18 and 21 mm zone respectively); while it was resistant

Table 1: Number and percentage prevalence of Bacterial species in raw beef, mutton and fish.

Raw meat samples	*Escherichia coli*	*Salmonella enteritidis*	*Staphylococcus aureus*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Shigella dysenteriae*
Beef (n=40)	18(45%)*	8(20%)#	12(30%)*	5(12.5%)	6(15%)*	4(10%)*
Mutton (n=40)	12(30%)#	7(17.5%)	10(25%)	4(10%)	4(10%)*	5(12.5%)*
Fish (n=20)	5(25%)	3(15%)	5(25%)	2(10%)	0(0%)	1(5%)
Total (n=100)	35	18	27	11	10	10

* Significantly higher than other meat types at $p < 0.05$.
# Significantly higher than fish meat at $p < 0.05$.

Table 2: Number and percentage prevalence of bacterial species in cooked beef, mutton and fish samples.

Cooked meat samples	*Escherichia coli*	*Salmonella enteritidis*	*Staphylococcus aureus*	*Bacillus cereus*
Beef (n=20)	5(25%)*	2(10%)	3(15%)*	2(10%)
Mutton (n=20)	5(25%)*	2(10%)	3(15%)*	2(10%)
Fish (n=10)	2(20%)	1(10%)	1(10%)	1(10%)
Total (n=50)	12	5	7	5

* Significantly higher than other meat types at $p < 0.05$.

Table: 3: Total bacterial count (g^1) in raw and cooked beef, mutton and fish meat.

Raw meat samples	Total bacterial count g^1	Cooked meat Samples	Total bacterial count g^1
Raw beef	$1.76\times10^{6*}$	Cooked beef	$6.1\times10^{4*}$
Raw mutton	$1.55\times10^{6\#}$	Cooked mutton	$4.5\times10^{4\#}$
Raw fish	1.25×10^{6}	Cooked fish	2.9×10^{4}

* Significantly higher than other meat types at $p < 0.05$.
# Significantly higher than fish meat at $p < 0.05$.

Table 4: Antimicrobial sensitivity of bacterial species isolated from raw and cooked fish, mutton and beef.*

Bacterial species (No. of isolates)	Antimicrobials (potency)**							
	GEN (10ug)	NOR (10ug)	N (30ug)	S (10ug)	AMP (20ug)	TET (30ug)	CIP (5ug)	ERY (15ug)
E.coli (n=47)	19mm (S)	18mm (S)	7mm (R)	3mm (R)	6mm (R)	2mm (R)	21mm (S)	4mm (R)
Salmonella Enteritidis (n=23)	17mm (S)	16mm (S)	8mm (R)	1mm (R)	6mm (R)	0mm (R)	16mm (S)	6mm (R)
Staphylococcus aureus (n=34)	18mm (S)	19mm (S)	7mm (R)	2mm (R)	5mm (R)	3mm (R)	22mm (S)	6mm (R)
Bacillus cereus (n=16)	20mm (S)	20mm (S)	7.5mm (R)	5mm (R)	6mm (R)	7mm (R)	18mm (S)	8mm (R)
Klebsiella pneumoniae (n=10)	20mm (S)	20mm (S)	0mm (R)	19mm (S)	13mm (I)	22mm (S)	17mm (S)	19mm (S)
Shigella dysenteriae (n=10)	21mm (S)	16mm (S)	0mm (R)	0mm (R)	12mm (I)	15mm (I)	5mm (R)	7mm (R)

* Zone of inhibition (mm) categorized as Susceptible (S), Intermediate (I) and Resistant (R) according to CLSI, (2012).
**GEN: gentamicin; NOR: norfloxacin; N: neomycin; S: streptomycin; AMP: ampicillin TET: tetracycline; CIP: ciprofloxacin; ERY: erythromycin.

against neomycin, streptomycin, ampicillin, tetracycline and erythromycin (7, 3, 6, 2 and 4 mm zone respectively). Our findings were closely related to Abd-El-Tawab et al. (2015) who stated that *E.coli* was highly sensitive to enrofloxacin, cefotaxime, gentamycin, and norfloxacin while ciprofloxacin was moderately sensitive and resistant to neomycin, streptomycin, ampicillin and oxytetracycline. Gamal et al. (2020) reported that *E.coli* were sensitive to norfloxacin, gentamycin, ciprofloxacin and florphenicol while resistant against oxytetracycline, amoxicillin, ampicillin, streptomycin and erythromycin.

S. enteritidis was found sensitive to gentamycin, norfloxacin and ciprofloxacin (17, 16 and 16 mm zone respectively), whereas, the organism was observed resistant against neomycin (8mm), streptomycin (1mm), erythromycin (6mm), ampicillin (6mm) and tetracycline (0mm). Our results were agreed with Abd-El-Tawab et al. (2015) who isolated foodborne bacteria from chicken and meat products in Kaliobia Governorate. He reported that *S. enteritidis* was highly sensitive to gentamycin, norfloxacin, enrofloxacin, ciprofloxacin, and cefotaxime; and weakly sensitive to neomycin, while resistant against erythromycin, ampicillin, streptomycin and oxytetracycline.

S.aureus showed sensitivity to norfloxacin (19mm), gentamycin (18mm) and ciprofloxacin (22mm); while the organisms was found resistant to neomycin (7mm), erythromycin (6mm), streptomycin (2mm), tetracycline (3mm) and ampicillin (5mm). Our results were in agreement with Gamal et al. (2020) who stated that *S.aureus* was highly sensitive to norfloxacin, gentamycin, ciprofloxacin and resistant against ampicillin, methicillin, oxytetracycline, streptomycin and erythromycin. Owuna et al. (2015) reported the high sensitivity against gentamycin, ciprofloxacin, erythromycin and amoxicillin.

B.cereus exhibited sensitivity against gentamycin (20mm), norfloxacin (20mm) and ciprofloxacin (18mm); however, *B.cereus* showed resistant to erythromycin (8mm), neomycin (7.5mm), streptomycin (5mm), ampicillin (6mm) and tetracycline (7mm). . Our results are agreed with Gamal et al. (2020) who reported that *B.cereus* isolates were susceptible to gentamycin, norfloxacin, and ciprofloxacin; while resistant against ampicillin, oxytetracycline, streptomycin, neomycin and erythromycin. Mousa et al. (2020) described antibiotic resistant *B.cereus* in Kaliobia Egypt and reported 82.3% sensitivity to both gentamycin and norfloxacin, 74.5% to ciprofloxacin and resistant against ampicillin, and oxytetracycline; whereas intermediate sensitivity showed against streptomycin, erythromycin and neomycin.

K. pneumoniae showed sensitivity against gentamycin (20mm), norfloxacin (20mm), ciprofloxacin (17mm), erythromycin (19mm), tetracycline (22mm) and streptomycin (19mm); whereas the organism was found intermediate to ampicillin (13mm) and resistant to neomycin (0mm). Our results were related to Oko et al. (2016) who reported that *K. pneumoniae* showed 91.67% sensitivity to ciprofloxacin, 66.67% to norfloxacin and gentamycin, 83.33% to erythromycin, 91.67% to chloramphenicol, cephalexin and 41.67% to tetracycline, 25% to ampicillin. Zhang et al. (2018) reported that the organism was highly susceptible to ceftazidime and piperacillin (100%), ciprofloxacin (93.6%), norfloxacin (95%), gentamycin (91.9%), streptomycin (82.2%), tetracycline (87.1%) while resistant against ampicillin.

S. dysenteriae was observed highly sensitive to gentamycin (21mm) and quite sensitive against norfloxacin (16mm). This organism showed intermediate sensitivity to both tetracycline (15mm) and ampicillin (12mm); while resistant to ciprofloxacin (5mm), erythromycin (7mm), neomycin and streptomycin (0mm). Our results were agreed with Goud et al. (2018) who reported that *Shigella* isolates were resistant against ampicillin, tetracycline, chloramphenicol, ciprofloxacin and erythromycin, but sensitive to gentamycin, co-trimoxazole, and ceftriaxone.

Increased use of antibiotics for treatment and prevention of microbial infections and growth promoters, are a risk factor for increasing bacterial resistance (Bogaard et al., 1997). Due to the indiscriminate use of antibiotics as therapeutics and prophylactic drugs, as well as growth promoters among animals, the treatment and control of foodborne infections is increasingly becoming difficult. In foodborne bacterial isolates, developing drug resistance is a major public health issues, thereby demanding the careful use of antimicrobial agents, particularly in veterinary medicine (Caprioli et al., 2000).

CONCLUSIONS

From the results of current investigation, it could be concluded that raw and cooked meat (fish, mutton and beef) were found contaminated by different bacterial species such as *E.coli*, *S. enteritidis*, *S.aureus*, *B.cereus*, *K. pneumoniae* and *S. dysenteriae*. Raw beef samples were found more contaminated than raw fish and mutton while cooked fish samples were observed less contaminated than cooked beef and mutton. The bacterial load was found higher in raw meat samples than cooked meat. All bacterial isolates (except *K. pneumoniae*) were found multidrug resistant. Contamination in raw meat (mutton, beef and fish) samples urge the health regulatory bodies to ensure hygienic practice in meat sector.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

NOVELTY STATEMENT

This is the first study reported from Hyderabad district that had explored the bacteriological quality of raw as well as cooked meat samples including beef, mutton and fish. The study data could be used by food regulatory authorities to formulate guidelines for local meat vendors in terms of public health.

AUTHORS CONTRIBUTION

SA carried out the experiments, SHA conceived the study, AJT and AS helped in statistical analysis and wrote the manuscript, GA and SA proofread the manuscript and helped in revision, and AAK supervised the study.

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