

Bacteriological Study of Fresh Market's Meat (Beef and Mutton) of Rawalpindi/Islamabad Region

Pages with reference to book, From 214 To 217

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Abstract

Six hundred samples, 300 each of mutton and beef were collected under aseptic conditions from meat shops of Rawalpindi/Islamabad. They were examined bacteriologically under aerobic and anaerobic conditions at different temperatures for varying periods of time. Different species of bacteria isolated were *Escherichia coli* (520), *Staphylococcus aureus* (437), *Staphylococcus epiderimidis* (200), *Streptococcus faecalis* (105), *Corynebacterium pyogenes* (50), *Bacillus subtilis* (84), *Salmonella typhi* (50), *Proteus mirabilis* (140), *Pseudomonas aeruginosa* and *Aerobacter aerogenes* (80). (JPMA 34 : 214, 1984).

Introduction

The study was carried out to find out the type of bacterial contaminants and their mode of action and proliferation during storage and transportation upto the disposal to the consumers under the prevailing conditions in twin city Rawalpindi/Islamabad.

Material and Methods

Three hundred each of fresh beef and mutton samples were collected from randomly selected animal carcasses at various meat shops of Rawalpindi/Islamabad areas.

Two samples, each weighing about 20 gms were taken from each carcass comprising of (i) inner portion of the muscles and (ii) external surface of the carcass. Each sample was taken in a clean sterilized wide mouth glass stoppered bottle using sterilized forceps and scissors. The samples were handled carefully to avoid contamination and transported to the laboratory for bacterial and cultural examination on selected media.

- a) Selenite - F - enrichment medium (Oxoid)
- b) Thioglycollate Broth (“)
- c) Cooked meat medium (“)
- d) MacConkey's agar (Difco)
- e) Brilliant green agar (“)
- f) Salmonella Shigella agar (Oxoid)
- g) Staphylococcus medium No. 110 (“)
- h) Eosin methylene blue agar (“)
- i) Triple sugar iron agar (“)
- j) Nutrient agar (“)
- k) Blood agar (10% defibrinated sheep blood plus Nutrient agar)

The following enrichment, selective, differential and normal culture media were used in this study:

Diagnostic Antigens and Anti Sera for Identification of Different Species of Organisms were

1. Rabbit serum -- used for coagulase test.
2. Diagnostic salmonella antisera (Difco)

Samples of meat (Beef and Mutton) were emulsified in sterile solvents like phosphate buffer or physiological saline alongwith sterile sand in pestle and mortar in perfect aseptic conditions. The homogeiate was allowed to stand for 10-15 minutes in order to get clear supernatant. One ml of supernatant was dispensed aseptically in three tubes, each containing liquid medium like, cooked meat medium, thioglycollate and selenite-Fenrichment medium. Shaked well and incubated, cooked meat medium tubes were incubated under anaerobic condition at 37°C for 24 hours, while Thioglycollate and Selenite-F-enrichment medium tubes were incubated under aerobic condition at 37°C for 24 hours. The growth in liquid medium was examined microscopically by gram staining and streaked on solid media like nutrient agar, blood agar and MacConkey's agar. The inoculated plates of nutrient agar and blood agar were incubated anaerobically at 35.37°C for 24.-48 hours, while three plates of each of solid media i.e. Nutrient agar, Blood agar and MacConkey's agar were incubated under aerobic conditions at 35-37°C, at room temperature (24-26°C) and at refrigeration temperature (4-10°C) for varying periods as shown in table 1. The morphology of representative colonies was studied from the plates and organisms were identified by biochemical reactions and slide agglutination tests using specific antisera. Coagulase positive Staphylococcus aureus were isolated on blood agar and identified by Pigment Production, mannitol fermentation and coagulase test.¹ Isolation of anaerobes was done using the method of Dowell et al².

Salmonellae were isolated according to the procedure described by Mildred et al³.

Results

From the bacteriological study of 600 meat samples, a total of 1841 isolates were obtained, of which 981 (53.28%) were gram-positive Cocci and Bacilli, where as 860(46.72%) were gram-negative rods of bacterial isolates, 1736 (94.30%) were aerobés while 105 Isolates (5.70%) were strictly anaerobes. The species identified were, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis, Clostridium Perfringens, Bacillus subtiis, Corynebacterium pyogenes, Salmonella typhi, Proteus mirabiis, pseudomonas aerugenosa, and Aerobacter aerogenes (Table I).

Table – I

Type and Number of Bacterial Isolates from Beef and Mutton Samples.

Type of isolates	Incubation		300 beef samples		300 mutton samples	
	Temperature	Time	No. of Isolates	% age	No. of Isolates	% age
Escherichia coli			270	90.00	250	83.33
Staphylococcus aureus			201	67.00	236	78.66
Staphylococcus epidermidis			80	26.00	120	40.00
Streptococcus faecalis	i) 35–37° Aerobically	(24–48 hrs)	50	16.00	55	18.33
Bacillus subtilis	ii) 24–26° (Room Temp.)		35	11.66	49	16.33
Corynebacterium Pyogenes			15	5.00	35	11.66
Proteus mirabilis			60	20.00	80	26.66
Aerobacter aerogenes			35	11.66	45	15.00
Salmonella typhi			20	6.66	30	10.00
Pseudomonas aeruginosa	4–10° (Refrigeration temp.)	(7 days)	35	11.66	35	11.66
Clostridium perfringens	35–37° (Anaerobically)	(24–48 hrs)	40	13.33	65	21.60

In the present study only 35 bacterial strains were isolated from the Inner portion of the muscles of meat samples, which were identified as Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa and Corynebacterium pyogenes.

Isolates identified from the surface meat samples of carcasses were, Escherichia Coli, Staphylococcus aureus, Staphylococcus epidermidis Proteus mirabiis, Streptococcus faecalis, Clostridium perfringens, Bacillus subtiia, Pseudomonas aeruginosa, Aerobacter aerogenes, Salmonella typhi and Corynebacterium pyogenes (Table II).

Table II

Distribution of Bacterial Strains Isolated from Beef and Mutton Samples collected
from various Portions of Animal Carcasses.

Source of SAMPLES	Escherichia Coli		Staphylococcus aureus		Staphylococcus epidermidis		Proteus mirabilis		Streptococcus faecalis		Clostridium perfringens		Bacillus subtilis		Pseudomonas aerogenes		Aerobacter aerogenes		Salmonella typhi		Corynebacterium pyogenes	
	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B	M	B
External surface of carcasses	205	270	230	199	120	77	80	60	55	50	65	40	49	35	30	33	45	35	20	18	30	15
Internal portion of muscles	0	0	6	2	0	3	0	0	0	0	0	0	0	0	5	2	0	0	10	2	5	0
Total	250	270	236	201	120	80	80	60	55	50	65	40	49	35	35	35	45	35	30	20	35	15

M = Mutton

B = Beef.

Discussion

Bacteriological study of 600 beef and mutton samples showed bacterial contamination with species of the genera, Escherichia, Staphylococcus, Streptococcus, Clostridium, Bacillus, Salmonella, Proteus, Pseudomonas, Corynebacterium and Aerobacter. The presence of these Isolates in the higher percentage in meat samples collected from the retail market of Rawalpindi/Islamabad areas is an indication of unsatisfactory handling of meat and inadequate hygiene. Most of bacterial contamination of meat occurs during post slaughter handling in the slaughter house and during transportation.⁴⁻⁶ Escherichia Coli was the main contaminant. Its incidence rate was 90% and 83.33% in samples of beef and mutton respectively. Escherichia coli was isolated only from the surface meat samples of carcasses and not from the inner portion of the muscles, which means that the source of entry of these organisms may be through polluted water and unhygienic handling of the slaughtered animals. Similar high incidence of Escherichia coli in raw meats has also been reported in other series.^{7,8} Organisms next in order of frequency were, Staphylococcus aureus (67% and 78.66%) and Staphylococcus epidermidis (26.66% and 40%) in case of beef and mutton samples respectively. These organisms are mostly present in raw meats due to surface pollution by polluted water and unsatisfactory hygienic conditions in the abattoirs.⁹ Prevalence of Streptococcus faecalis in meat is an indication of faecal pollution.^{10,11} Streptococcus faecalis can cause illness among humans through ingestion, which means raw meat handlers are at risk of infection by Streptococcus faecalis too.^{10,11} Of the anerobes only Clostridium Perfringens could be identified in 13 and 21% of beef and mutton samples respectively. Similar figures were reported in another study¹². Clostridium perfringens are very widely distributed, inhabiting soil and intestinal tract of animals. Its occurrence indicate that meat could have been contaminated with these two sources.

The incidence of all other isolates, the Salmonella typhi, Corynebacterium pyogenes, Bacillus subtiis, Proteus mirabilis, Pseudomonas aerugenosa, and Aerobacter aerogenes, ranged between 5 to 18%.

Salmonellae are important from public health point of view, being pathogenic microorganisms. They may be transferred to the carcass surface during slaughtering, handling and processing of meat.^{3,13,14} Species of the genera *Proteus* are constantly present in rotten meat, and sewage as well as human and animals excreta. Their incidence rate in meat is fairly high¹⁵. *Pseudomonas* is also a common psychrophile causing spoilage of meat^{15,17} This organism may warrant attention especially when meat is refrigerated.

Most of the microorganisms in the present study (1806 of 1841) were isolated from the surface meat samples of carcasses, while internal portion of the muscles yielded only 35 bacterial strains. Isolation of such a high number of bacterial strains from surface meat samples have also been made by other workers.^{16,17} Since only 35 isolates were identified from the inner portion of the muscle of meat samples, it is obvious that inner muscle portion are relatively clean.^{10,18} The frequency of bacterial contamination of meat in present study is quite high. Species of the genera, *Escherichia*, *Proteus*, *Aerobacter* and *Pseudomonas* are important in respect to their capabilities lot spoilage of meat and meat products at temperature varying between 4 to 37°C, whereas species of the genera, *Salmonella*, *Clostridium*, *Corynebacterium*, *Staphylococcus* and *Bacillus subtilis* are pathogenic and capable of producing disease through toxin production or direct infection through meat handling and processing. Thus consumption of highly polluted meat even after cooking appears to be unsafe due to heat stable toxins and spore bearing organisms.

Acknowledgement

The help and guidance of Dr Abdul Ban, Dept of Biological Sciences, Quaid-e-Azam University Islamabad in carrying out this work, Mr Zafar Mi, National Institute of Health, Islamabad in preparation of manuscript is gratefully acknowledged.

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