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Isolation of bacteria of public health significance from market beef

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Abstract

Food safety is a matter of increasing concern among consumers of meat especially with reference to food borne microbes. In India a major section of the population consumes meat derived from one or more species of food animals. In most of the unauthorised slaughter house, the stunning, sticking, bleeding, flaying and evisceration operations are carried out on the ground at a single place in the abattoir. Such practices lead to microbial contamination of meat from the exterior of the animal and the intestinal tract. In the present study 100 beef samples were collected from major meat stalls located at East Fort (EF), West Fort (WF), Sakthanthampuran Market (SM) and Mannuthy (MN) areas of Thrissur district in Kerala. All the samples collected from the four retail areas were tested for the isolation and identification of *Escherichia coli*, Salmonella , *Staphylococcus aureus* and *Listeria monocytogenes.* None of the samples yielded *Listeria monocytogenes* and Salmonellae, but 82 per cent samples had *E coli*. The serotypes obtained were O3,O19,O22,O25,O29, O34, O36, O42, O50, O51, O53, O55, O65, O66, O73, O79, O105, O109, O115, O139, O140, O147, O152, O163, O164 and O173.Two per cent of the samples had coagulase positive Staphylococci.

Keywords: *E. coli*, *L. monocytogenes*, *S. aureus*, Salmonella, beef.

Introduction

Food safety is a matter of increasing concern among consumers of meat especially with reference to food borne microbes. In India a major section of the population consumes meat derived from one or more species of food animals. The average per capita consumption of meat in India is 5kg per capita per year but that of Kerala is 20 Kg which is four times the country's average (Binsy *et al.*, 2014). A major chunk of the Keralite population is non-vegetarian, relishing meat delicacies. About 82.8 per cent of Keralites consume at least one non-vegetarian dish every day (Wilson, 2010). Meat has a high water content corresponding to the water activity approximately 0.99 which is conducive for bacterial growth (Rao *et al*., 2009). In most of the unauthorized slaughter house, the stunning, sticking, bleeding, flaying and evisceration operations are carried out on the ground at a single place in the abattoir. Such practices lead to microbial contamination of meat from the exterior of the animal and the intestinal tract . A number of bacterial organisms belonging to different genera are associated with food borne infection and intoxication in human beings. Meat can be contaminated with bacterial pathogens of animals such as *Escherichia coli*, Salmonella, *Listeria monocytogenes*, *Staphylococcus aureus* and Campylobacter species .Many of these organisms can be transmitted to meat and its products from food handlers and also from contaminated environment, equipment and utensils. Thus evaluation of the

microbial quality of meat is of great importance in determining the bacterial quality, wholesomeness and the hygienic practices followed during the production processing, distribution and retailing of meat. Considering the above facts, the present study was undertaken to isolate *Escherichia coli*, Salmonella, *Staphylococcus aureus* and *Listeria monocytogenes* from market beef in Thrissur, Kerala.

Materials and Methods

A total of 100 beef samples were collected from major meat stalls located at East Fort (EF), West Fort (WF), Sakthanthampuran Market (SM) and Mannuthy (MN) areas of Thrissur district in Kerala. Samples were bought were being sold to customers. From each of the above areas, 25 samples were collected and every sample weighed 500g. Immediately after collection, all samples were brought to the laboratory in thermo cool containers for the estimation of *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Listeria monocytogenes.*

Preparation of the sample

From each sample an initial test sample of about 100 g was prepared by cutting approximately five millimetre thick meat from the exterior and cut surface of the sample. These pieces were further cut into very small pieces so as to form the initial test sample.

Processing of sample

In order to estimate the various bacterial loads per gram of sample, 25 g sample was weighed from the initial test sample and transferred into sterile polythene bags containing 225 ml of sterile normal saline solution so as to form one in 10 dilution and agitated using a stomacher.

Escherichia coli

To isolate *E. coli* a loopful of the inoculum from the one in 10 dilution of every sample was inoculated onto duplicate plates of EMB agar. The plates were incubated at 37 ^{0} C for 24 h. Four or five colonies with dark centre giving distinct indelible ink, greenish black metallic sheen on deflected light was transferred onto nutrient agar slants from each sample. The inoculated slants were incubated at 37^{0}C overnight and stored at refrigeration temperature for further characterization and identification. The isolates were subjected to biochemical tests (Barrow and Feltham, 1993).The positive isolates were serotyped at National Salmonella and Escherichia centre, Central Research Institute, Kasauli, Himachal Pradesh.

Salmonella

From the initial test sample, 25 g was weighed and transferred into a sterile conical flask containing 225 ml tetrathionate broth (Hi-media).The contents of the flask were mixed using a cyclomixer at low speed for three minutes to get uniform suspension of the sample. The sample was incubated at 37 ^{0}C for 48h. Similarly another 25 g sample was transferred into a sterile conical flask containing 225 ml selenite cystein broth (Hi-media) and was treated identically as that of tetrathionate broth sample and incubated at 43^0 C for 48 h.

At 24 and 48 h of incubation, a loopful from each broth was inoculated onto duplicate plates of Brilliant Green agar (BGA) (Hi-media) and was incubated at 37^{0}C for 24 h. At the end of incubation, colourless, pink white opaque to translucent colonies with a diameter of one to two millimetre, surrounded by a pink or red hue were selected and transferred onto nutrient agar slants and incubated at 37^{0}C overnight. These slants were stored at refrigeration temperature for further characterization and identification of the isolates. The isolates were subjected to biochemical tests as per Barrow and Feltham (1993).

Staphylococcus aureus

To isolate *S. aureus*, a loopful of inoculum from the one in 10 dilution of each sample was inoculated onto duplicate plats of Tellurite Polymixin Egg Yolk Agar (TPEYA) (APHA, 1976).The plates were incubated at 37^0 C for 24 h. Four or five black circular convex colonies having a diameter of 1 to 1.5 mm with a zone of precipitation around the colony or colonies with a clear zone of halo around them and a white precipitate beneath the colony were transferred onto nutrient agar slants from each sample. The inoculated slants were incubated at 37^{0}C overnight and stored at refrigeration temperature for characterization. Every isolate was subjected to the biochemical test (Barrow and Feltham, 1993).

Listeria monocytogenes

The method described by Wang (1992) was followed for the isolation of the organism. From the initial test sample, 20 g of meat was transferred to a sterile conical flask containing 100 ml Listeria enrichment broth (Hi-media).The contents of the flask were mixed

using a cyclomixer at low speed for three minutes in order to get a uniform suspension of the sample. The inoculated sample was incubated at 30^0C for seven days. At the second and seventh day of incubation, loopful of inoculum was streaked onto duplicates plates of Oxford (Hi-media) and Polymyxin B Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol (PALCAM) agar (Hi-media) plates. The plates were incubated at 30^0 C for 24 to 48h.Colonies showing characteristics of aesculin hydrolysis in both plates were streaked onto Lithium chloride Phenylethanol Moxalactam (LPM) agar (Hi-media) and incubated at 30^0C for 24 h. At the end of incubation, plates were observed under Hendry's oblique lighting technique for further characterization. Greyish-blue typical bacterial bodies were picked and streaked onto soy agar slants and incubated at 30^0C for 24 h and stored at refrigeration temperature for further characterization. The isolates were subjected to the various tests prescribed by Kobuko (1990).

Results and Discussion

All the samples collected from the four retail areas were tested for the isolation and identification of *Escherichia coli*, Salmonella, *Staphylococcus aureus* and *Listeria monocytogenes*.

Escherichia coli

Escherichia coli is one of the mesophilic commensal organisms found in the gastro intestinal tract of man and animals. In humans, pathogenic strains of *E.coli* are responsible for intestinal diseases (gastroenteritis) and extra intestinal infections, which include urinary tract infections (UTI), bacteremia, and neonatal meningitis *E.coli* accounts for more than 90% of all uncomplicated UTIs (Fratamico *et al*., 2009).From the 100 samples of beef examined, 82 per cent had *E. coli*. The number of samples that revealed the presence of *E. coli* from the four areas is shown in table 1.The organism was present in 92 per cent of samples from WF area and 72 per cent of the samples from the EF area. However, the organism could not be isolated from two (8 per cent), four (16 per cent), five (20 per cent) and six (24 per cent) samples from WF, SM, MN and EF, respectively.

Table1. Percentage isolation of *Escherichia coli* from the four areas

Retail Area	No. Tested	No. of samples with *E. coli*	Per cent
EF	25	18	72
WF	25	23	92
SM	25	21	84
MN	25	20	80

Serotyping

A total of 82 *E. coli* isolates were serotyped at National Salmonella and Escherichia centre, Central Research Institute, Kasauli, Himachal Pradesh. The serotype and number of isolates from each area is given in table 2. Of the 82 isolates, 75 fell into 26 serotypes, four were untypable and three were rough types. The serotypes O42 and O50 were isolated from samples belonging to the four retail areas and the former serotype constituted the highest number of organisms isolated.

The isolation of 26 serotypes indicated the level of contamination of the samples and also the divergent sources of contamination. The serotypes O173 and O42 had been isolated from samples belonging to the four retail areas. The isolation of four strains of enteroinvassive *E. coli* belonging to serotypes O152 and O164 (Altwegg and Bockemuhl,1998) from one or more samples collected from EF and WF areas indicate its public health significance since these serotypes were associated with diarrhoea in adults and children. The isolation of the serotype O25 is significant because the serotype causes infantile diarrhoea, septicaemia and urinary tract infection in man .The serotype O55 is associated with infantile diarrhoea was also isolated from one sample in WF area. O22 was the most frequent isolated serotype from diarrhoeagenic kid, calves and lambs (Pachaury and Kataria, 2012) which was also isolated from three samples in WF and one sample from SM areas.

Table 2. Distribution of the serotypes obtained from the four areas

Serotypes	No. of isolates from				Total
	EF	WF	SM	MN	
O3	-	-	-	1	1
O19	-	-	1	-	1
O22	-	3	1	-	4
O25	-	-	1	-	1
O29	1	1	-	2	4
O34	-	1	-	-	1
O36	-	1	-	-	1
O42	3	2	4	1	10
O50	-	1	1	4	6
O51	2	1	1	-	4
O53	-	-	-	1	1
O55	-	1	-	-	1
O65	1	2	-	-	3
O66	1	-	1	-	2
O73	-	2	2	1	5
O79	1	-	2	1	4
O105	2	-	1	1	4
O109	-	1	1	-	2
O115	-	-	1	2	3
O139	1	1	-	2	4
O140	1	-	-	-	1
O147	-	-	1	1	2
O152	1	-	-	-	1
O163	-	-	1	-	1
O164	1	2	-	-	3
O173	2	1	1	2	4
Untypable	1	1	-	2	4
Rough	-	2	1	-	3

Staphylococcus aureus

S. aureus is one of the organisms commonly implicated in food borne intoxication. During the investigation, 98 per cent of the samples were found free from the organism however only two samples obtained from MN retail area had coagulase positive Staphylococci. Contrary to the findings of this study, Lubna *et al*., (2015) reported that 50 per cent of the beef samples tested were positive for the organism.

Salmonella

All the samples obtained from the four retail areas were found to be free from Salmonella. The finding corroborates with the study of Kalyapure *et al.,* (1994) and Venkateswaran *et al*., (1988).

Listeria monocytogenes

Cent per cent samples were found to be free from *L. monocytogenes*. Similar observations were made by Wang *et al.*, (1992).On the contrary Dimic *et al.*, (2010) isolated the organism from 77 per cent of beef tested.

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