Original article

Bacteriological quality of sheep meat in Mhow town of India

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Summary The purpose of this study was to investigate bacterial load in ready-to-sale sheep meat with special reference to *Salmonella*. Samples were collected from 100 sheep carcasses from retail meat shops in domestic markets. On carcasses, where bacterial counts were obtained, the mean of the $\log_{10}$ aerobic plate count was 7.26 cfu g^{-1}, and that of total coliform count and total *Escherichia coli* count was 4.11 $\log_{10}$ cfu g^{-1} and 3.03 $\log_{10}$ cfu g^{-1}, respectively. All the samples (100) were found positive for coliforms, 49.0% were positive for *E. coli* and 3.0% were positive for *Salmonella*. The isolates were serotyped as *Salmonella infantis* having antigenic structure 6, 7: r: 1, 5. Antibiogram revealed highest (100.0%) sensitivity towards amikacin, ceftriaxone, ciprofloxacin, chloramphenicol, colistin sulphate, gentamicin and nalidixic acid followed by cefuroxime and tetracycline (66.67% each) and cotrimoxazole (33.33%). All the strains were resistant to ampicillin.

Keywords Antibiogram, bacterial count, mice, *Salmonella infantis*, sheep meat.

Introduction

Meat arriving at the retail markets is contaminated with inordinate number of bacteria. Therefore, carcasses with lowest possible microbial load should be obtained, as microbes have direct bearing on the shelf life of fresh meat and bacteriological quality of the meat products.

The microbial quality of sheep meat has not been studied sufficiently, despite the fact that lamb is consumed in considerable quantities in many parts of the world, especially in the Middle and near East countries (Sajida *et al*., 1982). Most of the sheep meat produced in India is sold fresh in the retail market where meagre attention is paid towards hygiene. Extensive review of literature reveals that not much work has been done in India on the bacteriological quality of sheep meat. The product quality and standards that are followed in India, at present, are based on the findings of foreign workers, which are not appropriate for various reasons such as variation in climatic conditions, socioeconomic status of producer and consumer, differences in the animal husbandry practices and subsequent slaughtering and processing of the animals. Hence, if any standards are to be laid, the guidelines should be set in such a way that they confirm to the prevailing conditions in the country and at the same time ensure the high quality of the product. Hence, survey of the present bacteriological status of sheep meat is of immense importance for rationalising the standards of the sheep meat and its products in India.

In its demand for animal proteins, humanity is often exposed to food-borne infections and intoxications. Every year millions of people are affected by food-borne diseases and thousands die of it. Improper methods of food production, storage, handling and preparation have resulted in many recognised international outbreaks. Globalisation of trade has further facilitated the spread of food-borne infections and diseases between distantly placed nations. Accordingly, foods of animal origin need to be closely monitored during production as well as during handling, processing and distribution (Anon., 2002).

India also has a good export potential for meat owing to its huge livestock population. However, failure to maintain the internationally accepted hygienic and microbial standards has resulted in ban on import of Indian meat by several countries in the past (Chander, 2003). Among various health hazards, food-borne bacteria, especially those of non-typhoidal *Salmonella*, are most important and common. Hence, the present study was undertaken to assess the level of *Salmonella* contamination in fresh sheep meat with special reference to the serotypes, pathogenicity and antibiogram.

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Materials and methods

This study was conducted at different retail market shops of sheep meat, located in Mhow town of Madhya Pradesh (India). One hundred samples were taken from randomly selected five retail market shops on every Sunday. Not more than five samples were taken at a time, one each from five different meat shops. Microbiological examination was carried out within an hour after the collection of samples.

The samples were collected as per the procedure recommended by International Commission on Microbiological Specification for Food (ICMSF, 1978). Samples were collected by excision method (destructive method) taking approximately 10 g samples from each of the five sites, viz. neck, right forelimbs and hind limbs and left forelimbs and hind limbs (Amin & Borah, 2002). These samples were transferred to the laboratory in sterile stomacher bags on ice. Samples that were above 10 °C upon arrival at the testing laboratory were discarded.

Samples were thawed to room temperature. The sample collected from five different sites of each carcass was pooled in one stomacher bag. The pooled sample was then homogenised at 8000 r.p.m. for 2 min in 450 mL of normal saline solution (NSS) to obtain 1:10 dilution. Subsequently, tenfold serial dilution up to 10^{-10} were carried out in sterile NSS (ICMSF, 1978). This 1:10 diluted sample was used as an inoculum for determination of *Salmonella*.

In the present study, aerobic plate count (APC), total coliform count (TCC) and total *Escherichia coli* count (TEC) of each pooled sheep carcass sample were enumerated according to the method described by ICMSF (1978) with minor modifications. For enumeration purpose, from each tenfold dilution, 0.1 mL was transferred with the help of sterile pipettes on a sterile media plate. The inoculum was then spread by a spreader (hockey-stick-like glass rod). For each dilution triplicates of appropriate media and different sets of sterile pipettes and spreader were used (Table 1). The inoculated plates were kept at room temperature for 30 min to allow the inoculum to be adsorbed and then incubated at 37 °C for 24 h. The bacterial colonies were counted using digital colony counter (Model No. 361; M/s Environmental and Scientific Instrument Co. Pvt. Ltd, India).

For calculation of counts (APC, TCC and TEC), the mean count of three plates was multiplied with the dilution factor (Prasad & Yadava, 2000). All counts were converted to log values to enable statistical analysis based on the assumption of normal distributions. Data were statistically analysed as per the method suggested by Snedecor & Cochran (1994).

Table 1 Media used for counts

Count	Media	Company
APC	Plate count agar (PCA)	M/s Biomark
TCC	Violet red bile agar (VRBA)	Laboratories Pvt. Ltd,
TEC	Eosin methylene blue (EMB) agar	Pune, India

The 1:10 diluted sample was used as initial inoculum for the detection of *Salmonella*. The samples were pre-enriched in 10 mL buffered peptone water (pH 7.0 ± 0.1) by inoculating 1 mL of 1:10 diluted sample and incubating for 48 h at 37 °C. After pre-enrichment, 1 mL of inoculated buffered peptone water was transferred into 10 mL of modified Rappaport–Vassiliadis (MRV) medium (M/s Biomark, Pune, India) and incubated at 37 °C and 43 °C for a maximum period of 7 days. A loopful from enriched MRV medium was inoculated onto brilliant green sulpha agar and desoxycholate citrate agar (DCA) (M/s Biomark) at 37 °C for 24–48 h to obtain well-isolated colonies.

The isolated colonies on selective media were identified as *Salmonella* on the basis of their cultural, morphological and biochemical characteristics (Barrow & Feltham, 1993). These were confirmed serologically by slide agglutination test using anti-*Salmonella* polyvalent 'O' (A–G) and H antisera. The confirmed *Salmonella* isolates were sent for serotyping at the National *Salmonella* Typing Centre, Central Research Institute (CRI), Kasauli.

The isolates that were confirmed as *Salmonella* after serotyping were used for pathogenicity testing, which was determined by inoculating 0.1 mL of 18-h old broth culture (ca $\sim 3 \times 10^8$ viable bacteria per mL) of each isolate into separate adult Swiss albino mice by intraperitoneal route; two mice per isolate were used. The inoculated mice were observed up to 96-h postinoculation and those that died were necropsied and examined for gross lesions. Impression smears were prepared from visceral organs and heart blood of dead mice and stained by Gram's method to demonstrate bacteria. To prove Koch–Henle's postulates, attempts were also made to reisolate Salmonellae from heart blood on DCA.

To test production of toxin(s) by the isolates, procedure of Giannella (1976) was adopted with minor modifications. Each bacterial strain was cultured in brain–heart infusion (BHI) broth at 37 °C for 18 h in shaker incubator (G-24; American Laboratory Trading, New Brunswick, NJ, USA) at a speed of 200 r.p.m. Subsequently, broth culture was centrifuged at 12 298***g***. for 45 min at 4 °C in a refrigerated centrifuge (C-23; Remi, Mumbai, India) to sediment the bacterial cells. The supernatant was aseptically collected, passed through a membrane filter (F-25DB Laxbro, Millipore, Pune, India) of 0.22 μm diameter porosity and stored at −20 °C until use. The toxic effect of this cell free filtrate (CFF) was tested by inoculating 0.1 mL in adult healthy Swiss albino mice by intraperitoneal route. Two mice

per strain were used and one mouse was kept as control in which equal volume of sterile BHI broth was injected. The mice were observed up to 96-h postinoculation and those found dead were necropsied and examined for gross lesions.

The sensitivity of isolates towards eleven different antibiotics was determined by *in vitro* disc diffusion method of Bauer *et al.* (1966) on Muellor–Hinton agar (M/s BioMark). The drugs used for sensitivity testing included ampicillin (10 mcg), amikacin (30 mcg), ceftriaxone (30 mcg), cefuroxime (30 mcg), colistin (30 mcg), chloramphenicol (10 mcg), ciprofloxacin (05 mcg), cotrimoxazole (1.25/23.75 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg) and tetracycline (30 mcg). The discs were purchased from M/s Hi Media Labs (Mumbai, India). The results were interpreted according to the instructions of the manufacturer.

Results

Bacteriological quality of sheep carcasses from Mhow town of India is summarised in Table 2. A total of 100 samples representing the bacteriological quality of sheep carcasses were collected from five different local shops. Mean $\log_{10}$ APC for sheep carcasses was 7.26, maximum and minimum being 7.51 and 7.04, respectively. Mean $\log_{10}$ TCC for sheep carcasses was 4.11, maximum and minimum being 4.28 and 3.78, respectively. Mean $\log_{10}$ TEC for sheep carcasses was 3.03, maximum and minimum being 3.36 and 2.78, respectively.

Salmonellae were isolated from 3.0% sheep meat samples. In present study Salmonellae were isolated following enrichment at 43 °C than at 37 °C. However, delayed enrichment up to 7 days did not increase the isolation rate of *Salmonella*. Biochemical characterisation of all the three isolates confirmed majority of reactions to be that of typical Salmonellae. However, variation was recorded in utilisation of Simmon's citrate medium and rhamnose, only 66.67% isolates were positive for these tests. Similarly, contrary to standard results, none of the strain fermented inositol, while all utilised dulcitol, maltose, sorbitol and xylose and produced H_2S gas.

All the three strains were typed as *Salmonella infantis* having antigenic structure 6, 7: r: 1, 5.

Table 2 Bacterial counts per gram of sheep meat

	Aerobic plate count ($\log_{10}$ g^{-1})	Total coliform count ($\log_{10}$ g^{-1})	Total *Escherichia coli* count ($\log_{10}$ g^{-1})
Minimum	7.04	3.78	2.78
Maximum	7.51	4.28	3.36
Average	7.26 ± 0.01	4.11 ± 0.01	3.03 ± 0.02
Percentage positivity	100	100	49

Pathogenicity testing of bacterial culture as well as CFF of all the three isolates resulted in death of mice within 12-h postinoculation, whereas control mice survived even after 96-h postinoculation. The gross lesions observed were acute gastroenteritis with haemorrhages and accumulation of fluid in gut. The bacteria also caused septicaemia with congestion of internal organs. Impression smears revealed the presence of Gram-negative coccobacilli. *Salmonella* was reisolated from the heart blood of dead mice confirming Koch–Henle's postulates.

The *in vitro* antibiotic sensitivity test of *Salmonella* strains revealed 100% susceptibility towards amikacin, ceftriaxone, ciprofloxacin, chloramphenicol, colistin sulphate, gentamicin and nalidixic acid. All the three isolates recovered from sheep meat were resistant to ampicillin; whereas 66.67% were sensitive to cefuroxime and tetracycline, and only 33.33% sensitive to cotrimoxazole.

Discussion

The present study is based on excised sheep carcasses. APCs were accepted as a useful hygiene indicator for the microbial contamination of carcasses and in combination with TECs as verification of slaughter hygiene (Mackey & Roberts, 1993). APC results reported in this investigation for sheep carcasses after destructive method of sampling ranged from 7.04 (11×10^6) to 7.51 (32×10^6) cfu g^{-1}. Comparable counts were reported in India by Borah *et al.* (1992) and Khurana & Kumar (1994), as 0.02×10^6 to 201×10^6 cfu g^{-1} and 3.2×10^4 to 1.5×10^6 cfu g^{-1}, respectively. However, on the contrary, Vanderlinde *et al.* (1999) in Australia and Zweifel & Stephan (2003) in Switzerland recorded lower APC count as 3.92 cm^{-2} and 2.5–3.8 cm^{-2}, respectively. However, comparison is difficult because of different sampling methods, different sampling sites, different hygienic standards followed in abattoirs and different geoclimatic conditions. According to Bureau of Indian Standards (BIS, 1995) the maximum permissible limit of APC in sheep meat, where meat should be rejected for human consumption, is 5×10^6 cfu g^{-1}. Thus, all the 100 samples examined in the present study had the counts higher than the proposed Indian standards. High APC can be dangerous for the consumers, as some of the microorganisms could be pathogenic (Berry & Foegeding, 1997).

High coliform count indicated poor hygienic quality of sheep meat. The contamination with coliforms may occur during slaughtering, cutting or dressing of carcasses, soiled hands, shopping blocks or knives used for handling and cutting or contaminated water are the important sources of coliforms in meat. Moreover, Bell *et al.* (1993) reported that high number of bacteria could be transferred from the fleece of sheep to the carcass

surface during hide removal. Bell & Hathaway (1996) reported that the areas of the highest contamination in carcasses were the sites where cuts are made through the skin.

The forty-nine carcass samples in the current study had detectable *E. coli* (less than 2 cfu g^{-1} was considered non-detectable). So the mean count of the *E. coli* was taken from detectable samples only. Higher count of *E. coli* from sheep meat suggests faecal contamination and contact with contaminated water or equipment to be more frequent at the site in particular and on the whole carcasses in general as a result of the slaughtering techniques used.

The incidence of *Salmonella* (3.0%) found on sheep carcasses might be due to the relatively high incidence and numbers of *E. coli*. Similar findings were also reported by Vanderlinde *et al.* (1999) who observed 5.74% samples positive for *Salmonella*. According to BIS (1995), sheep meat should be completely free from *Salmonella*. *Salmonella* may be carried on the fleece or in internal organs, thus cross contamination of meat may occur during defleecing or evisceration of carcasses.

In the present investigation, higher temperature (43 °C) during enrichment was found to favour the recovery of *Salmonella* over other enteric bacteria, which has earlier been reported by Siems *et al.* (1975). The recovery of atypical *Salmonella* from sheep meat, as observed in the present study, was in agreement with the findings of Shivhare *et al.* (2000) who also reported atypical *Salmonella* from animal and animal products. *Salmonella infantis* recovered in this study rank among the twenty most important *Salmonella* serotypes of public health significance, according to WHO report (Anon, 1988). Food poisoning as a result of the consumption of meat contaminated with *S. infantis* has been reported from different parts of the world (Kohl & Farley, 2000; Gonera, 2002). Thus, isolation of this serovar warrants adaptation of proper precautions.

Pathology of non-adopted Salmonellae and their CFF in mice as observed in this research pursuit was also recorded by Shivhare *et al.* (2000). *Salmonella* can readily penetrate intestinal mucosa causing acute septicaemia. It also elaborates number of toxins including heat-labile enterotoxin that leads to accumulation of fluid (Sedlock *et al.*, 1978). The results of *in vitro* antibiotic sensitivity test observed in this research pursuit were also reported by Sharma & Joshi (1992) and Bacon *et al.* (2002).

The high counts suggest that sheep marketed in the area under study is not fit for human consumption. Recovery of *Salmonella*, which is one of the important causes of food poisoning, is alarming and should be viewed seriously. The isolation of pathogenic strains suggest that either diseased or carrier animals were used for slaughtering, or the hygienic conditions in the slaughterhouses and meat shops were of very low standards. The production of such poor quality sheep meat predisposes it to early spoilage as well as a threat to the health of the consumer. The animals slaughtered for human consumption should be healthy and disease free. Hence, strict hygienic measures should be adopted during slaughtering, processing and transportation of meat and meat products for producing the end product of eatable quality.

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