

Prevalence of microorganisms of hygienic interest in an organized abattoir in Mumbai, India

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Abstract

Background: The magnitude of food-borne illnesses in India is unknown because of lack of surveillance networks. Monitoring the prevalence of food-borne pathogens and indicators of contamination in primary production at abattoirs is imperative for creating a data bank and for effective control of such pathogens before they enter the food chain.

Methodology: Microorganisms of hygienic interest were screened for their prevalence at Deonar Abattoir, Mumbai. Swab samples from 96 sheep/goat carcass sites were collected and analyzed for *Staphylococcus* spp., *Bacillaceae*, *Clostridiaceae* and *Enterobacteriaceae*.

Results: Average *Staphylococcus aureus* and *Staphylococcus epidermidis* counts were 3.15 ± 0.18 and $3.46 \pm 0.17 \log_{10}$ CFU/cm², respectively. *Bacillus cereus*, *Bacillus subtilis* and *Clostridium* spp. counts were 3.10 ± 0.08 , 3.41 ± 0.19 and $0.76 \pm 0.06 \log_{10}$ CFU/cm², respectively. The *Escherichia coli* count was 3.54 ± 0.06 and the *Klebsiella aerogenes* count was $3.22 \pm 0.22 \log_{10}$ CFU/cm². Counts for *Proteus vulgaris* and *Proteus mirabilis* were $3.44 \pm 0.14 \log_{10}$ CFU/cm² and $3.71 \pm 0.12 \log_{10}$ CFU/cm², respectively. *S. epidermidis* had the highest percentage prevalence at (41.6%), followed by *K. aerogenes* (31.9%), *B. subtilis* (28.2%) and *P. vulgaris* (23.6%). *Salmonella* spp. were not isolated.

Conclusions: The data demonstrate high prevalence and diversity of micro flora on carcasses in the primary Indian production facility, which might be attributed to either human handling or improper dressing especially during evisceration process. Appropriate training for personal and production hygiene is essential for workers in Indian meat production facilities.

Key words: microorganisms of hygienic interest; sheep/goat carcasses; abattoir; prevalence

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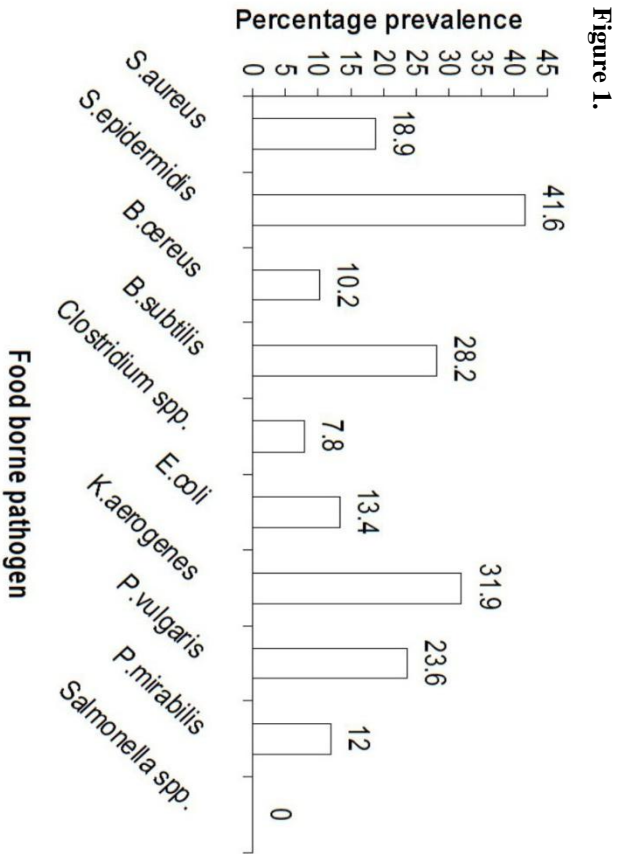
Introduction

Food-borne diseases are a threat to public health worldwide. The repercussions are not only health related but also have economic ramifications from the loss of business over food safety issues. In the United States of America (USA), 60% of cases requiring hospitalization are caused by the food-borne bacteria [1], but in developing countries related data is not available because of a lack of precise health-care infrastructure and data management. It is estimated that annually about 1.8 million children in developing countries succumb to death from acute diarrhoeal illnesses, with food or water being the major causes of many of these illnesses [2]. Chugh [3] has identified the emerging and reemerging zoonotic, food-borne and waterborne diseases as well as the diseases caused by multidrug resistant organisms as the major public health threats in India. However, the magnitude of food-borne illnesses in India is unknown due to the lack of a coordinated surveillance network.

As far as foods of animal origin are concerned, India is the largest milk producer in the world [4]; however, in spite of the potential from the large livestock population, meat production is below capacity but is building. The total meat processing capacity in India is over one million tons per annum, of which 40-50 percent is utilized [5]; thus there is tremendous scope in this sector. Given the present attention from the government and private entrepreneurs, the future of the meat industry in India looks prosperous; however, much work is needed in maintaining quality assurance to prevent food-borne diseases. Monitoring the prevalence of microorganisms of hygienic interest in primary production at abattoirs is imperative for creating a data bank and for effective control of such pathogens before they further enter the food chain. The present study was undertaken at an organized abattoir in Mumbai city to determine the prevalence of microorganisms of hygienic interest.

Table 1. Mean bacterial counts (log10 CFU/cm² ± SD) and percentage prevalence of various food-borne pathogens isolated from sheep/goat carcasses at Deonar Abattoir, Mumbai.

Sr. No	Food borne pathogen	After flaying	Percentage prevalence	After evisceration	Percentage prevalence	After washing	Percentage prevalence	Average counts	Average percentage prevalence
2	<i>S. aureus</i>	3.09 ± 0.11	13.9	3.24 ± 0.21	29.1	3.11 ± 0.21	13.8	3.15 ± 0.18	18.9
3	<i>S. epidermidis</i>	3.48 ± 0.16	33.3	3.67 ± 0.13	50.0	3.24 ± 0.22	41.6	3.46 ± 0.17	41.6
4	<i>B. cereus</i>	3.17 ± 0.10	11.1	3.04 ± 0.04	8.3	3.08 ± 0.11	11.1	3.10 ± 0.08	10.2
5	<i>B. subtilis</i>	3.41 ± 0.31	30.5	3.48 ± 0.15	29.1	3.34 ± 0.12	25	3.41 ± 0.19	28.2
6	<i>Clostridium</i> spp.	0.00 ± 0.00	0.00	2.27 ± 0.17	20.8	2.00 ± 0.00	2.7	0.76 ± 0.06	7.8
7	<i>E. coli</i>	3.55 ± 0.08	11.1	3.95 ± 0.06	20.8	3.11 ± 0.05	8.3	3.54 ± 0.06	13.4
8	<i>K. aerogenes</i>	3.05 ± 0.15	22.2	3.39 ± 0.30	45.8	3.23 ± 0.20	27.7	3.22 ± 0.22	31.9
9	<i>P. vulgaris</i>	3.32 ± 0.15	30.5	3.89 ± 0.18	29.1	3.10 ± 0.09	11.1	3.44 ± 0.14	23.6
11	<i>P. mirabilis</i>	3.69 ± 0.28	22.2	3.96 ± 0.06	8.3	3.49 ± 0.03	5.5	3.71 ± 0.12	12.0
12	<i>Salmonella</i> spp.	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.00	0.00 ± 0.00	0.0



Materials and methods

All the media used were dehydrated and purchased from HiMedia laboratories, Mumbai, India. Swab samples were collected from sites on sheep and goat carcasses after various slaughter operations (flaying, evisceration and washing) at an organised abattoir (*i.e.*, a modern, mechanised and export-oriented abattoir with proper infrastructure and skilled manpower to slaughter a large number of animals, which follows the safety specifications given by the Meat and Meat Product Order of 1973 issued by the Directorate of Marketing and Inspection, Government of India) at Deonar, Mumbai. A total of 96 samples were taken at the abattoir from six different carcasses on six occasions (Table 1) from the neck, shoulder, brisket, loin, flank and rump after flaying and washing. After evisceration samples were collected from the brisket and rib medial, brisket and rib lateral, and loin and flank regions. Carcass sites were sampled by the swab technique. Briefly, an area of 100 cm² was marked with a sterile frame of 10 cm x 10 cm for each site on the carcass. Sterile cotton wool swabs (3 cm long and 1 cm in diameter) held by wooden sticks moistened with 0.1% peptone were rubbed on the sites continuously for 30 seconds and transferred to a screw-capped test tube containing 10 ml of sterile maintenance medium (0.85% NaCl and 0.1% peptone). The screw-capped test tubes were brought to the laboratory in a thermos flask containing ice and processed immediately.

Test tubes containing swabs were shaken on a vortex mixer for 30 seconds for uniform distribution of microorganisms. Tenfold serial dilution of all the samples was prepared using sterile normal saline solution (NSS). Then the samples were processed for viable counting.

Food-borne organisms were enumerated using various selective and differential media employing spread or pour plate techniques with 0.1 ml inoculum from 10⁻² and 10⁻³ dilutions decided on the basis of the results of pilot studies used to pre-estimate the microbial load at each abattoir. Differential count plates were incubated for 24-48 hours and, with the exception of some media for Clostridia and *Salmonella*, were incubated at 37°C. Vogel Johnson plates were used for isolation and enumeration of *Staphylococcus* spp. Egg yolk agar and blood agar were used for isolation and enumeration of *Bacillus* spp. and *B. cereus* isolation agar (Code M833) was used for isolation and identification of *B. cereus* in suspected cases. *Clostridium* species were isolated by wax overlay

anaerobic technique upon Sodium Polymyxin Sulphadiazine (SPS) agar incubated at 44°C for 24 hours.

Various members of the family Enterobacteriaceae were isolated and enumerated using MacConkey agar with crystal violet. Eosin Methylene Blue agar was employed for isolation and identification of *E. coli* showing typical metallic sheen. To isolate *Salmonella* spp. swab samples were homogenised in 225 ml buffered peptone water and pre-enriched at 37°C for 24-48 hours; then 1 ml of culture was transferred to 10 ml of Selenite Cystine broth (enrichment medium) and incubated at 44°C for 18 hours. Selective plating was done on Brilliant Green Sulpha agar and incubated 43°C for 24 hours.

Two to three characteristic colonies of each bacterium were further subjected to purification, identification, and characterization. Microscopic examination was conducted on smears stained by modified Gram's staining as described by Beveridge [6]. Characterisation and identification of organisms was done according to Barrow and Feltham [7] and Cheesbrough [8]. All counts were converted to log₁₀ CFU/cm² along with standard deviation and percentage prevalence was calculated.

Results

The results obtained are shown in Table 1 and Figure 1. The *Staphylococcus* spp. isolated were *Staphylococcus aureus* and *Staphylococcus epidermidis* with their average counts as 3.15 ± 0.18 and 3.46 ± 0.17 log₁₀ CFU/cm², respectively. From the family Bacillaceae, *Bacillus cereus* and *Bacillus subtilis* were isolated with their average counts as 3.10 ± 0.08 and 3.41 ± 0.19 log₁₀ CFU/cm², respectively. The counts of *Clostridium* spp. from family Clostridiaceae were 0.76 ± 0.06 log₁₀ CFU/cm². Organisms from the Enterobacteriaceae family that were isolated include *Escherichia coli*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Proteus mirabilis*, but *Salmonella* spp. *E. coli* and *K. aerogenes* were recovered at 3.54 ± 0.06 and 3.22 ± 0.22 log₁₀ CFU/cm², respectively, while counts of *P. vulgaris* and *P. mirabilis* were 3.44 ± 0.14 and 3.71 ± 0.12 log₁₀ CFU/cm², respectively. It was noticed that the differential counts were increased after the evisceration process and were reduced after washing of the carcasses (Table 1).

The highest percentage prevalence (Figure 1) noticed was that of *S. epidermidis* (41.6%), followed by *K. aerogenes* (31.9%), *B. subtilis* (28.2%), and *P. vulgaris* (23.6%). Lower percentage prevalences

were seen for *Clostridium* spp. (7.8%), *B. cereus* (10.2%) and *P. mirabilis* (12%). The prevalence of *S. aureus* was 18.9%, while that of *E. coli* was 13.4%.

Discussion

The present data represent the prevalence of a diversity of microflora on carcasses in the primary Indian production facility. Similar to our study, Narasimha Rao and Ramesh [9] observed average counts for *Staphylococcus* spp. as $1.63 \log_{10}$ CFU/cm². Borse *et al.* [10] also isolated *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp. and *Enterobacter* spp. as predominant flora in sheep carcasses and Vijaya Rao *et al.* [11] recovered *Staphylococcus* (44.6%), *Enterobacteriaceae* (9.2%) and *Bacillus* (4.2%) as predominant organisms from fresh mutton. McDowell *et al.* [12] reported similar microflora at brisket, forequarters and rear-quarters with 8% *Staphylococcus aureus*, 13% other *Staphylococci*, and 14% *Bacillus* spp. Krustch [13] isolated *Clostridium* spp. from 4% swab samples of sheep carcass surfaces, which is almost in accordance with the observations of the present study. Sierra *et al.* [14] found *E. coli* as the main pathogen in swabs taken from lamb carcasses, while *Enterobacter*, *Klebsiella* and *Serratia* spp. were detected in lower numbers. Also, Biss and Hathaway [15] found *E. coli* from 21.37% of lamb carcass surfaces.

The high incidence of *Staphylococcus* spp. in this study is indicative of a contamination due to human handling as these organisms are the commensals of human skin and mucosal surfaces. Bendahou *et al.* [16] mention that the enterotoxin-producing *S. aureus* can be dangerous and harmful to human health. Similarly, other major prevalent *Enterobacteriaceae* family organisms such as *K.aerogenes*, *B.subtilis* and *P. vulgaris* are also inhabitants of the respiratory and intestinal tract of humans as well as animals. Thus their prevalence may be attributed to either human handling or improper dressing, especially during the evisceration process. The infections caused by such Gram-negative bacteria are a worldwide public health problem [17]. Per European Commission [18] norms, *Enterobacteriaceae* counts greater than $3.0 \log_{10}$ CFU/cm² are unacceptable but the present study shows higher counts than the prescribed limits even after the washing operation.

It is encouraging that *Salmonella* spp. could not be detected in this study. *Salmonella* is a common cause of bacterial food-borne gastroenteritis in many countries worldwide and in India it is known to be endemic [19]. The overall annual incidence of food-

borne salmonellosis in India is nearly 6 per 1,000 inhabitants [20]. The most commonly implicated serotypes for disease outbreaks are *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype Enteritidis [21]. Non-typhoid salmonellosis originating from foods of animal origin is an emerging infection in India and is noted to be an increasing trend [22].

Although the hazard analysis critical control point system (HACCP) is implemented at the Deonar Abattoir in its sheep/goat unit, with the exception of the absence of *Salmonella* spp., the occurrence of the other aforementioned microorganisms of hygienic interest is a serious concern. To evaluate the effectiveness of the food safety programs, baseline studies are needed to determine levels of indicator organisms and prevalence of pathogens [23]. The farm-to-fork approach is advised in food safety management and detection of such food-borne pathogens and indicators of contamination at an abattoir makes it necessary to also look back at the farms to reduce the microbial load. India does not have organized farming practices for meat production with appropriate veterinary input; thus it is difficult to receive the animals with least microbial load at the lairages. Management practices such as pre-slaughter fasting at lairages with access to water, along with the maintenance of strict hygiene and sanitation at the slaughterhouses, can help to reduce the microbial load of carcasses. Appropriate training for personal and production hygiene is highly essential for the people working in Indian meat production facilities.

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