Enumeration and Antibiotic Resistance Pattern of
S. aureus from Raw Chevon Meat Sold in Bikaner City

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A total of 50 chevon samples were collected from various meat shops of Bikaner city (Rajasthan) to examine the presence of **Staphylococcus aureus** and study antibiotic sensitivity pattern of isolated *S. aureus*. 46 (92%) samples were found to be contaminated with average counts of *S. aureus* 4.347 log 10 cfu/g. Half of the observed values to be in the potentially hazardous category. Among these 46 *S. aureus* isolates, 21 (45.65%) were coagulase positive. The results of antibiotic sensitivity tests revealed all *S. aureus* isolates (100%) sensitive to ciprofloxacin and doxycycline. In contrast, all isolates of *S. aureus* (100%) were resistant to ampicillin and cloxacillin. Moreover, *S. aureus* isolates were highly sensitive to gentamycin while most of the isolates were highly resistant to tetracycline and ofloxacin. Multidrug resistance was also found in most of the isolates. This study revealed high prevalence of *S. aureus* in raw chevon samples and also evidenced antibiotic resistance of the organism. The study reflects the poor hygienic condition of slaughtering and handling of chevon meat.

Key words: *Staphylococcus aureus,* Goat meat/chevon, antibiotic sensitivity tests, multidrug resistance.

Among meats, goat meat (chevon) is one of the common dietary items for the people of our country consuming meat. It has for long occupied a special place in the diet as a source of animal protein for a variety of reasons including taste preference, prestige, religion, tradition and availability for almost all the communities (Dhanda, 2001).

Meat along with its nutritious value is highly susceptible to microbial contaminations, which can cause its spoilage and food borne infections in human, resulting in economic and health losses (Komba *et al.*, 2012). In this row, *Staphylococcus aureus* is one of the most common causes of food borne infections in majority of countries (Bergdoll, 2007). Various virulence factor of *Staphylococcus* have been found to be responsible for several animal diseases including suppurative disease, mastitis, arthritis and urinary tract infections. In humans, the organism is a major cause of food poisoning, pneumonia, postoperative wound infections and nosocomial bacteremia (Sidhu *et al.*, 2007). *S. aureus* are normal inhabitant of skin and mucous membrane of animals and human (Norrung, *et al.*, 2009) presence of *S. aureus* or its enterotoxins in processed foods or on food processing equipment is generally an indication of poor sanitation and hygienic

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condition (Adesiji Yemisi *et al.*, 2011; Eze and Ivuoma, 2012).

Bacterial antimicrobial drug resistance is another worldwide problem and the effectiveness of currently available antibiotics is decreasing due to increasing number of resistant strains (Nawaz *et al.*, 2009). The indiscriminate and inappropriate use of antibiotics has led to emergence of antibiotic resistant strains (Alian et al., 2012; Yurdakul *et al.*, 2013). Antibiotic resistant staphylococci are major public health concern since the bacteria can be easily circulated in the environment. *S. aureus* resistant to antibiotics have been identified in various foods (including meat) in large population during recent years (Myles and Datta 2012).

Since, traditional ways of slaughter lacking hygiene are commonly being used in Bikaner; there is a need for proper evaluation of meat before supply for human consumption. Determination of level of *S. aureus* could serve as a tool for determining hygienic standard implementation and the antibiotic sensitivity test gives idea about resistant strains of *S. aureus*. A few studies have been done on chevon in this direction. Hence, keeping all the facts in mind the present study was planned with the objective of enumeration of *S. aureus* from raw chevon, to conduct coagulase test in isolated *S. aureus* and to study antibiotic sensitivity pattern on isolated *S. aureus*.

MATERIALS AND METHODS

Collection of the meat samples
Raw chevon samples (n=50) were collected from various meat shops of Bikaner city. All possible precautions were taken to collect samples aseptically and the samples were immediately brought to the laboratory under cold conditions for further processing.

Enumeration of *Staphylococcus aureus*
For enumeration of staphylococci; the surface spread method on MSA medium recommended by NCFA (1968) was used. The colonies showing fried egg appearance with golden yellow or orange or cream colour were considered *Staphylococcus* spp. and counting was expressed in log colony forming unit per gram (log cfu/g). To examine the typical suspected colonies of

Staphylococcus spp. Gram's reaction, catalase test, oxidase test were conducted. Identified colonies were further subjected to coagulase test described by Cowan & Steel (1975).

Antibiotic Sensitivity Test (ABST)
Antibiotic sensitivity test (ABST) was conducted as per the procedure prescribed by Blazevic *et al.* (1972). Ten antibiotic discs *viz.*, Ampicillin, Chloramphenicol, Ciprofloxacin, Cloxacillin, Doxycycline, Erythromycin, Gentamicin, Kanamycin, Ofloxacin and Tetracycline were selected for conducting the sensitivity tests.

RESULTS AND DISCUSSION

Enumeration of *Staphylococcus aureus*
High prevalence rate of *S. aureus* in raw goat meat was found in the present study as out of 50 chevon samples 46 (92%) were found positive for *S. aureus*. Similarly, Haque *et al.* (2008) also found the presence of *S. aureus* in all raw goat meat samples collected during their study. In some other studies lower numbers of goat meat samples were positive for *S. aureus*. Rahimi *et al.* (2013) tested 80 chevon samples, out of which 38 (47.5%) yielded *S. aureus*. Mukhopadhyay *et al.* (2009) and Adesiji Yemisi *et al.* (2011) found only 21.74% and 12% goat samples positive for *S. aureus*. *S. aureus* counts from all the positive samples of chevon in the present study ranged between 2.602 to 6.812 with an average count of 4.347 log 10 cfu/g. Ahmad *et al.* (2013) and Singh *et al.* (2014) in their investigation reported lower *S. aureus* counts in goat meat. In another study Haque *et al.* (2008) found the values 3.96 log 10 cfu/g, 3.37 log 10 cfu/g and 4.22 log 10 cfu/g in brisket, neck and thigh regions of goat meat, respectively. Very small quantities of staphylococcal enterotoxins (less than 1 μg) is responsible for illness in humans and these levels of toxin are generally observed when *S. aureus* populations exceed 10^5 CFU/g of food (FDA, 2012). In the present study in 24 out of 46 positive samples (52.17%) *S. aureus* populations exceeded 10^5 CFU/g. So, presence of *S. aureus* in such a high number in the present investigation could be potentially hazardous for chevon meat consumers as even after cooking *S. aureus* toxins may have deleterious effects on consumer's health. Similarly, Meat handlers are also at risk of getting *S. aureus* infection.

The high prevalence of *S.aureus* in meat indicates poor hygienic and working practices of the meat handlers during the processing stage (Cohen *et al.*, 2007; Adetunji and Odetokun, 2011). It may be due to cross contamination usually related to human skin, nasal passage and clothing, since, *Staphylococcus* spp. is a part of normal flora on the skin of humans and animals and transmissible from person to product through unhygienic practices (Postgate, 2000). Similarly, in the present study there may be several factors responsible for the high isolation rate of *S. aureus*. One of the observations made during the sampling was that, in Bikaner traditional ways of slaughter are followed for meat production and most of the meat is sold by unorganized retailers where standard practices of slaughtering are not followed. Polluted environment and contaminated equipments at the slaughter places and meat shops could be another source of contamination. All the slaughtering practices being done in the same place without any sanitary measures expose the final product to all sorts of contamination. In addition, it was found that slaughtered carcasses were hanging uncovered in open places which make the meat more prone to contamination by wall surface, dust, dirt and microbial air pollutants. Hansson (2001) and Spescha *et al.* (2006) have also reported that different stages of slaughter affect the prevalence and bacterial load of *S. aureus* on the carcass. The unscientific practices followed for slaughtering in the present study as discussed above could be the reason for half of the observed values to be in the potentially hazardous category as per the standards laid down by FDA.

Test for coagulase production

In this study, among 46 *S. aureus* isolates, 21 (45.65%) were found coagulase positive, whereas, 25 (54.34%) did not show coagulation and were considered coagulase negative *S. aureus*. The results were not at par with the findings of Prasad and Yadav (2000) who detected 50 per cent coagulase positive *S. aureus* strains in raw chevon meat in their study. Coagulase enzyme production by *S.aureus* is considered a criterion for determining its pathogenicity and is evaluated by coagulase test. Pathogenic strains are usually coagulase-positive and have been found to be responsible for diseases in their hosts throughout the world (FDA, 2012; FSANZ, 2013).

Antibiotic Sensitivity Test (ABST)

The results of antibiotic sensitivity test for all the *S. aureus* isolates were interpreted according to the literature supplied by the manufacturer (HiMedia Laboratories) and are shown in Table-1.

In the present study no resistance was found in *S. aureus* against ciprofloxacin similar to the findings of Heo *et al.* (2008) who did not record resistance in *S. aureus* from poultry meat. Although Otalu *et al.* (2011) had observed some degree of resistance towards the ciprofloxacin in poultry meat. In contrary, all the *S. aureus* isolates were found resistant to ampicillin in the current study. Whereas, Heo *et al.* (2008) reported 50 per cent of *S. aureus* isolates resistant to ampicillin in their study. Kelman *et al.* (2011) found *S. aureus* isolates from different meat samples were highly sensitive to ampicillin.

Table 1. Antibiotic resistance profile of *S. aureus* isolated from chevon

S.No.	Antibiotic agent	Resistance	Intermediate resistance	Susceptible
1.	Ampicillin	46 (100%)	0 (0%)	0 (0%)
2.	Chloramphenicol	18 (39.13%)	8 (17.39%)	20 (43.47%)
3.	Ciprofloxacin	0 (0%)	0 (0%)	46 (100%)
4.	Cloxacilllin	46 (100%)	0 (0%)	0 (0%)
5.	Doxycycline.	0 (0%)	0 (0%)	46 (100%)
6.	Erythromycin	6 (13.04%)	10 (21.75%)	30 (65.21%)
7.	Gentamicin	2 (4.34%)	4 (8.69%)	40 (86.95%)
8.	Kanamycin	18 (39.13%)	10 (21.73%)	18 (39.13%)
9.	Ofloxacin	37 (80.43%)	4 (8.69%)	5 (10.86%)
10.	Tetracycline	40 (86.96%)	3 (6.52%)	3 (6.52%)

S. aureus isolates showed high sensitivity against gentamicin in our results. The effectiveness of gentamicin towards *S. aureus* isolates was found better by Kelman *et al.* (2011). Moreover, Heo *et al.* (2008) recorded this antibiotic completely effective in their studies. But, Otalu *et al.* (2011) found more degree of resistance (38.5%) towards this antibiotic.

The present findings of antibiotic sensitivity test of chloramphenicol and erythromycin were in partial agreement with the results of *S. aureus* isolates of pork and chicken meat reported by Lin *et al.* (2009) who found these antibiotics more effective. Yurdakul *et al.* (2013) in their study on chicken meat found 25 per cent *S. aureus* isolates resistant to chloramphenicol and erythromycin, both. Although, Heo *et al.* (2008) did not find resistant *S. aureus* isolates from poultry in their study against chloramphenicol.

The antibiotic sensitivity pattern for tetracycline in the present study were not in accordance to the study by Lin *et al.* (2009) and Kelman *et al.* (2011) who observed lesser percentage (66.7% and 69% respectively) of resistant isolates. On the other hand, Heo *et al.* (2008) found greater per cent of resistant isolates as compared to present study, while Otalu *et al.* (2011) and Yurdakul *et al.* (2013) observed all isolates of *S. aureus* resistant to tetracycline in their study.

Multidrug-resistance which has been defined as resistance to 3 or more antimicrobial agents was found in most of the isolates in the present study. In another study (Waters *et al.* 2011) multidrug resistance was common among *staphylococcus* isolates (52%). Multidrug-resistant *S. aureus* strains are especially one of the greatest public concerns since the treatment of infections is more difficult when encountering resistance (Normanno *et al.*, 2007; Heo *et al.*, 2008).

Meat may serve as a reservoir of antibiotic resistant bacteria that can be transferred to humans, thereby constituting a public health problem (Iroha, *et al.*, 2011). The finding of a large number of *S. aureus* resistant to ampicillin, cloxacillin, tetracycline and ofloxacin in this study is of considerable concern as these drugs are commonly used in veterinary medicine in India. The indiscriminate use of antimicrobials has evoked serious bacterial resistance and emergence of new and highly resistant strains of bacteria to commonly used antimicrobials (Harakeh *et al.*, 2006) especially, in developing countries like India where widespread and uncontrolled use of antibiotics is there.

The present study indicates high prevalence of *S. aureus* in raw chevon meat in Bikaner and suggests a great need to educate meat handlers and sellers as well as consumers on the adverse effect of lack of proper personnel hygiene and sanitation. The study also provided important information regarding to antimicrobial resistance of *S. aureus* in raw chevon meat. More studies are required in this direction for designing treatment regimens as well as controlling the emergence of antimicrobial resistant *S. aureus.*

ACKNOWLEDGMENTS

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