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Enumeration and Antibiotic Resistance Pattern of *Staphylococcus aureus* from Raw Chicken Meat Sold in Bikaner City

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The present study was aimed to enumerate *Staphylococcus aureus* in raw chicken samples sold in Bikaner city (Rajasthan) and to study antibiotic sensitivity pattern of isolated *S. aureus*. For this a total 50 chicken samples were collected from various meat shops out of which 48 (96%) samples were found to be contaminated with *S. aureus*. *S. aureus* counts from all the positive samples ranged between 2.699 to 6.732 log 10 cfu/g, with average counts of 4.587 log 10 cfu/g. Out of these 48 isolates 20 (41.66%) were found coagulase positive. Antibiotic sensitivity test for the isolates revealed that all isolates of chicken meat samples (n = 48) were sensitive to ciprofloxacin and doxycycline (100%). *S. aureus* isolates were also found highly sensitive to gentamycin. In contrast, all isolates of *S. aureus* (100%) were resistant to ampicillin and cloxacillin while most of the isolates were also highly resistant to tetracycline. Multidrug resistance was also found in most of the isolates in the present study. The study revealed high prevalence of *S. aureus* in raw chicken meat samples and also evidenced antibiotic resistance of the organism. The study reflects the poor hygienic condition of slaughtering and handling of chicken meat.

Key words: *Staphylococcus aureus*, Chicken meat, Antibiotic sensitivity tests, Multidrug resistance.

Poultry meat is largely popular meat throughout the world that contains large amount of minerals and vitamins and has balanced lipid content with low caloric value. Attractiveness of poultry meat is also due to relatively low and competitive prices and absence of cultural or religious obstacles as compared to other meats[1]. Contaminated raw or undercooked poultry and red meats are particularly important in transmitting food-borne pathogens[2] and subsequently food borne diseases[3-4].

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Staphylococcal food poisoning is one of the most important causes of food borne diseases[5]. In India, rate of infection is higher owing to warm and humid climate[6]. Although, most staphylococci occur as commensals and are normal inhabitant of skin and mucous membrane of animals and human being; however, *Staphylococcus aureus* strains producing various toxins and enzymes are responsible for diseases in animals and humans[7]. Various virulence factor of *Staphylococcus* are associated with severe 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[8]. *S. aureus* can easily contaminate

chicken meat and this contamination is usually associated with a high presence of virulence and antibiotic resistance genes[9]. Rapid evolution of antibiotic resistance in *S. aureus* is of considerable concern due to complications in treatment. Multidrug-resistant *S. aureus* strains are especially, one of the greatest public health concerns since the treatment of infections is more difficult[10-11].

Poor hygienic process and direct contact with infected materials during the production process may lead to *S. aureus* contamination in meat[12]. Determination of level of *S. aureus* could serve as a tool for determining hygienic standard implementation during meat production. Since, traditional ways of poultry slaughter are commonly used in Bikaner which having poor hygienic condition and sanitation, so there is a need for proper evaluation of meat before supply for human consumption. Hence, keeping all the facts in mind present study was aimed with the objective of enumeration of *S. aureus* from raw chicken meat and study antibiotic sensitivity pattern of isolated *S. aureus.*

MATERIALS AND METHODS

Collection of the meat samples

Raw chicken 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 *S. aureus*

For enumeration of staphylococci; the surface spread method on MSA medium recommended by NCFA[13] 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[14].

Antibiotic Sensitivity Test (ABST)

Antibiotic sensitivity test (ABST) was conducted as per the procedure prescribed by Blazevic *et al.*,[15]. 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*

The results of present study showed high prevalence of *S. aureus* in chicken meat. 48 (96%) chicken samples were found positive for *S. aureus* out of total 50 samples collected. In other studies, Javadi and Safarmashaei[16] and Ahmad *et al*.[17] isolated *S. aureus* from 65 per cent and 55 per cent poultry meat samples in their respective studies, showing a relatively lower prevalence of organism. On the contrary, Normanno *et al*.[10] and Cohen *et al*. [18] reported only 10 per cent meat samples positive in their studies. *S. aureus* counts from all the positive samples of chicken ranged 2.699 to 6.732 log 10 cfu/g with average counts of 4.587 log 10 cfu/g in the present study. Ahmad *et al*.[17] in their investigation found lower *S. aureus* counts figuring 3.08 log 10 cfu/g in chicken. Higher *S. aureus* counts corresponding to 4.795 log 10 cfu/g and 5.36 log 10 cfu/g have been reported by Javadi and Safarmashaei[16] and Amara *et al*.[19] respectively. The permissible limit for *S. aureus* notified by Bureau of Indian Standards (BIS) is 3 log10 cfu/g[20]. In the present study, only 12 (24%) out of 50 chicken samples were found to fall in acceptable category. People become ill after exposure to very small quantities of staphylococcal enterotoxins (less than 1 µg). These levels of toxin are generally observed when *S. aureus* populations exceed 10^5 CFU/g of food[21]. In the present study in half of the positive samples (50%) *S. aureus* populations exceeded 10^5 CFU/g. So, presence of *S. aureus* in such a high number in this study could be potentially hazardous for chicken 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[18,22]. In this direction several factors may be responsible in the present study. 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 at the slaughter places and meat shops could be another source of contamination. Most of the retail meat shops sampled had live birds near the slaughter places, the butchers eviscerate the birds on the chopping block and make regular cuts of meat on the same chopping block. It has been reported to be a potential cause for contamination in earlier studies also[23]. The splashed blood and intestinal contents adhering to the chopping block were cleaned with the feathers of the slaughtered bird itself and in the last all the offals were discarded in the waste bin placed near it. This further enhances the chances of cross contamination of uninfected carcass by a previously cut infected carcass and also buildup of infection. All the slaughtering practices being done at the same place without any sanitary measures expose the final product to all sorts of contamination. Since, *S. aureus* is a part of normal flora on the body of humans and animals[24], the unscientific practices followed for slaughtering as discussed above could be the reason for most of the observed values. The presence of *S. aureus* in chicken meat samples indicates its possible contamination from the skin, mouth or nose and clothing of the workers handling the meat and inadequately cleaned equipment and surfaces[25].

Test for coagulase production

Out of 48 isolates from chicken samples, 20 (41.66%) were found coagulase positive and 28 (58.33%) were coagulase negative in the present study. In another study on refrigerated chicken rinse, Islam *et al.*[26] found 68.53% samples were coagulase positive Staphylococcus and 31.46% were negative. Likewise, Coagulase positive *S. aureus* was isolated from 54% of the samples by Suleiman *et al.*[27]. 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[21, 28, 29].

Antibiotic Sensitivity Test (ABST)

In the present study, antibiotic sensitivity tests were conducted against all 48 *S. aureus* isolates obtained from raw chicken samples. 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.

Table 1. Antibiotic resistance pattern of *S. aureus* isolates from chicken

S.No.	Antibiotic agent	Resistance	Intermediate resistance	Susceptible
1.	Ampicillin	48 (100%)	0 (0%)	0 (0%)
2.	Chloramphenicol	17 (35.42%)	8 (16.66%)	23 (47.92%)
3.	Ciprofloxacin	0 (0%)	0 (0%)	48 (100%)
4.	Cloxacilllin	48 (100%)	0 (0%)	0 (0%)
5.	Doxycycline.	0 (0%)	0 (0%)	48 (100%)
6.	Erythromycin	11 (22.91%)	7 (14.58%)	30 (62.50%)
7.	Gentamicin	3 (6.25%)	5 (10.41%)	40 (83.33%)
8.	Kanamycin	18 (35.50%)	10 (20.83%)	20 (41.66%)
9.	Ofloxacin	42 (87.50%)	3 (6.25%)	3 (6.25%)
10.	Tetracycline	39 (81.25%)	5 (10.41%)	4 (8.33%)

The cent per cent susceptibility of isolates to ciprofloxacin is in complete agreement to the findings of Heo *et al.*,[11] who did not record resistance in *S. aureus* from poultry meat. Although Otalu *et al.*,[30] had observed some degree of resistance towards the antibiotics in poultry meat where he found 84.6 per cent isolates sensitive to the antibiotic. In the current study, 100 per cent *S.*

aureus were found resistant to ampicillin similar to Islam *et al.*,[26] whereas in earlier studies Heo *et al.*,[11] reported 50 per cent of *S. aureus* isolates from poultry meat samples resistant to ampicillin. Further, only 17 per cent isolates from different meat samples were reported resistant to ampicillin by Kelman *et al.*,[31].

Results of this study also represent high sensitivity of isolates towards gentamicin. The effectiveness of gentamicin towards *S. aureus* isolates was found better by Kelman *et al.*[31] and Islam *et al.*[26] who recorded this antibiotic effective against most of the *S. aureus* isolates in their study. Moreover, Heo *et al.*,[11] recorded this antibiotic completely effective in their studies. But, Otalu *et al.*[30] found more degree of resistance (38.5%) towards this antibiotic in comparison to the present study.

The present findings of antibiogram towards chloramphenicol and erythromycin was in partial agreement to the results of *S. aureus* isolates of pork and chicken meat reported by Lin *et al.*[32] who found these antibiotics more effective. Yurdakul *et al.*[33] in their study on chicken meat found 25 per cent *S. aureus* isolates resistant to chloramphenicol and erythromycin, both. Although, Heo *et al.*[11] did not find resistant *S. aureus* isolates from poultry in their study against chloramphenicol.

The antibiotic sensitivity results for tetracycline in the present study were not in accordance to the study by Lin *et al.*[32] and Kelman *et al.*,[31] who observed lesser percentage (66.7% and 69% respectively) of resistant isolates. On the other hand, Heo *et al.*[11], Momtaz *et al.*[9] and Otalu *et al.*[30] found greater per cent of resistant isolates as compared to present study as they observed 92.9, 97.56 and 100 per cent *S. aureus* isolates resistant to tetracycline, respectively. Yurdakul *et al.*[33] also found all strains of *S. aureus* were resistant to tetracycline in their study on chicken meat.

Meat may serve as a reservoir of antibiotic resistant bacteria that can be transferred to humans, thereby constituting a public health problem [34]. The finding of a large number of *S. aureus* resistant to ampicillin, cloxacillin, tetracycline and ofloxacin 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[35]. 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[36] 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[10,11], 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 chicken meat in Bikaner city and suggests a great need to educate meat handlers and sellers as well as consumers on the adverse effect of lack of proper sanitation and personnel hygiene. The study also provided important information regarding to antimicrobial resistance of *S. aureus* in raw chicken meat. More studies are required in this direction for designing treatment regimens as well as controlling the emergence of antimicrobial resistant *S. aureus.*

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