Prevalence and antibiogram of *Staphylococcus aureus* isolated from foods of animal origin

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**Abstract**

The present study was conducted in a total of 120 samples collected from different parts of Udaipur city, Rajasthan in order to isolate and identify the *S. aureus* from milk, egg and meat samples. In total, 40 samples each of raw milk, egg and meat were processed for the isolation and identification of *Staphylococcus aureus* employing standard biochemical assays. All the samples were collected randomly from dairies, egg outlets and meat outlets located in different parts of Udaipur city, Rajasthan. Out of 120 samples analyzed, 19.17% (23/120) samples were found to be positive for *S. aureus*. Prevalence of *S. aureus* in raw milk, egg and meat samples was 37.5% (15/40), 5% (2/40) and 15% (6/40), respectively. In the investigation, all the isolates were subjected to antibiotic susceptibility test by using 12 different antibiotic discs. The analyses of antibiogram revealed that majority of the isolates were sensitive for amoxycil (73.91%),ampicillin (56.52%) and gentamicin (56.52%). However, the isolates revealed higher resistance for mitchellin (60.86%), co-trimoxazole (52.17%), oxytetracycline (52.17%) and streptomycin (52.17%).

**Keywords:** *Staphylococcus aureus*, raw milk, egg, meat, antibiotic susceptibility

**Introduction**

Food borne diseases are one of the major cause of concern in developing countries resulting in several deaths annually along with billions of dollars of economic burden. According to World Health Organization (WHO), approximately 600 million people are getting infected and around 420,000 die annually worldwide due to food borne illness. Most cases of food borne outbreaks are caused by Salmonella, Campylobacter, Escherichia coli, Listeria, Vibrio cholera and *Staphylococcus aureus* which have been reported worldwide [1]. Milk is a highly valuable food, but raw milk favors the growth of many microorganisms [2] and has also been reported as valuable medium for the growth of many microorganisms including pathogenic bacteria [3]. Due to non-judicious use of antibiotics in animal husbandry to treat infections and to improve growth and feed efficiency of animals, antimicrobial resistance has become a public threat. Other factor posing serious threat to the people is the rapid development of drug resistance in food borne pathogens. The use of antibiotics for therapeutics may lead to the development of drug resistance in microorganisms and later these resistant microorganisms can enter in to the food chains to cause severe human infections [4].

**Materials and Methods**

A total of 120 samples comprising of milk (n=40), egg (n=40), and meat (n=40) were collected from different areas of Udaipur city. The samples were collected aseptically in sterile sampling vials and transported on ice packs to the laboratory under chilled condition. After collection of samples, 1ml/1gm of the milk, egg and meat sample was inoculated in 9 ml of peptone water and incubated at 37 °C for 24 hrs. Then, a loopful of inoculum was streaked on selective media i.e. mannit salt agar (MSA) and incubated at 37 °C for 24 hrs. After 24 hrs, the plates were observed for the presence of yellow coloured colonies.
Morphology of individual organisms was characterized microscopically using Gram’s staining procedure according to the method described by Merchant and Packer (1967) [26]. Biochemical tests were performed to confirm the *S. aureus* using catalase test, oxidase test, coagulase test and haemolysis production (Mamza et al, 2016) [25].

All the confirmed, *Staphylococcus aureus* isolates were subjected to antibiotic sensitivity test as described by Bauer et al. (1966) [3]. To conduct antibiotic sensitivity test, 12 antibiotic discs (HiMedia) were employed viz., amoxycillin, ampicillin, ceftriaxone, chloramphenicol, co-trimoxazole, enrofloxacin, erythromycin, gentamicin, methicillin, oxytetracycline, polymixin-B and streptomycin. The diameter of the zone of inhibition were measured and the results were interpreted according to the CLSI guidelines.

**Results and Discussion**

In the present study, out of 120 samples, the prevalence of *S. aureus* was recorded in milk, egg and meat samples as 37.5% (15/40), 5% (2/40) and 15% (6/40), respectively. The test culture showing yellow coloured colony on MSA was selected for the further identification. All the isolates which were morphologically identified as Gram positive cocci arranged in grape-like clusters were selected and processed further.

All the isolates when mixed with a drop of 3% hydrogen peroxide over a clean glass slide showed the production of gas bubbles or effervescence within a few seconds and were designated as catalase positive. Similarly, all the isolates when applied on the surface of oxidase disc, exhibited the absence of colour development or delay in appearance of any colour and were considered as oxidase negative. The isolates were stabbed in motility agar medium and incubated at 37 °C for 24 hours. After incubation, the tubes were observed with growth along the line of inoculation. This indicated that all the isolates were non motile. An isolated colony when streaked on the 5% sheep blood agar and incubated at 37 °C for 24 hours showed complete haemolysis. Based on the cultural and biochemical tests, 23 isolates were confirmed as *S. aureus*.

In the raw milk samples, 37.5% (15/40) samples were contaminated with *S. aureus*, which was comparable with the findings of previous studies in India. Sudhanthiramani et al. (2015) [11] reported 39.09% prevalence of *S. aureus* in the milk samples. Similarly, Bharathy et al. (2015) [9], found 40% prevalence rate in raw milk sample from different regions of Chennai city. In another study, Wang et al. (2018) [6] evaluated the prevalence of *S. aureus* from milk in which out of the 195 raw milk samples, 90 (46.2%) were confirmed as *S. aureus*. Whereas, Tessaema and Tsegaye (2017) [9] found 28.2% of *S. aureus* in raw milk sample which was lower than the rate observed in the present study.

The prevalence of *S. aureus* obtained in the egg samples was 5%, which was in accordance with the earlier observations as conducted by Pondit et al. (2018) [10] exhibiting prevalence of 9%. Higher prevalence rate was revealed in the studies conducted by Bencardino et al. (2017) [11] which illustrated prevalence of 52%. While, a lower prevalence rate was also recorded for *S. aureus* contamination in egg by Rahman et al. (2018) [12].

Further, the prevalence of *S. aureus* in meat samples was 15% (6/40). Saleh et al. (2016) [13] reported prevalence of *S. aureus* as 22% which was similar to the prevalence observed in the present study. While, higher prevalence of *S. aureus* in meat samples was revealed by Shylaja et al. (2018) [14] and Herve and Kumar (2017) [1] as 73.33% and 46.61%, respectively. Similarly, Rahman et al. (2018) [12] and Thangaiavel and Anandhan (2015) [15] observed a lower prevalence of *S. aureus* as 2.56% and 10% respectively.

Further, in the present investigation, 12 different antibiotics were used to obtain antibiotic susceptibility pattern for 23 isolates of *S. aureus* recovered from milk, egg and meat samples. Out of the 15 isolates of *S. aureus* recovered from market milk, the analysis of antibiotic resistance revealed that the most resistant antibiotics were methicillin and polymixin-B (40% each) followed by ceftriaxone (33.33%). Out of the 2 isolates of *S. aureus* recovered from egg samples, chloramphenicol, co-trimoxazole, enrofloxacin, erythromycin, methicillin and Oxytetracycline showed highest resistance (100%) followed by polymixin-B (50%). While, out of the 6 isolates of *S. aureus* recovered from meat samples, chloramphenicol, co-trimoxazole, enrofloxacin, erythromycin, methicillin, oxytetracycline and streptomycin showed highest resistance (100%) followed by polymixin B (83.33%).

Similar findings were reported by Sharma et al. (2018) [16], who have reported resistance in *S. aureus* against methicillin, oxytetracycline and erythromycin. Rahman et al. (2018) [12], Gulzar et al. (2018) [17], and Jahan et al. (2015) [18] have reported highest resistance of *S. aureus* isolates towards erythromycin. While, Wang et al. (2018) [16] have reported intermediate sensitivity to erythromycin. Shrivastava et al. (2018) [19], Adetutu et al. (2017) [20] and Yadav (2018) [21] have also reported high resistance towards co-trimoxazole. Thaker et al. (2013) [22] and Adetutu et al. (2017) [20] reported high resistance of *S. aureus* isolates towards streptomycin. Most of the *S. aureus* isolates analyzed in our study were found to be susceptible to gentamicin and amoxyclav. Similar findings of *S. aureus* being sensitive to gentamicin were also reported by Rahman et al. (2018) [12], Ema et al. (2018) [23] and Ojadjare and Ekakene (2016) [24].

**Conclusion**

Milk, egg and meat are the major component in human diet. Milk serve as good medium for growth of *S. aureus*, so proper treatment of milk should be done before consumption. The difference in the level of prevalence that has been reported in different studies may be attributed to the variation in the sample size and geographic location. Presences of *S. aureus* in meat indicate poor hygiene and working practices of meat handlers during production and processing. Eggs are considered safe but the unhygienic condition of poultry farm poses a risk to human health. *S. aureus* is a facultative anaerobe and can survive in h...

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done to overcome the problem of antibiotic resistance in future.

References