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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 amoxyclav (73.91%), ampicillin (56.52%) and gentamicin (56.52%). However, the isolates revealed higher resistance for methicillin (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 (WHO, 2015) [7].

Milk is a highly valuable food, but raw milk favors the growth of many microorganisms (Helena *et al.*, 2010). Milk and its derivatives are considered as a major source of *Staphylococcus aureus* infection in man (Zecconi and Piccinini, 2000) [8]. Lack of sanitary conditions and improper microbiological monitoring of food are some of the major contributing factors responsible for the increase in the number of food borne infections. Meat, milk and egg are key component in human diet. Milk is important food for vegetarian class and has also been reported as marvelous medium for the growth of many microorganisms including pathogenic bacteria (Ruegg, 2003) [2]. 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 (Herve and Kumar, 2017) [1]

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. mannitol 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 haemolysin 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*., amoxyclav, 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 ^{0}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 ºC 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) [4] reported 39.09% prevalence of *S. aureus* in the milk samples. Similarly, Bharathy *et al.* (2015) [5], 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, Tessema 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 Thanigaivel 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 polymyxin-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 polymyxin 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) [6] 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 Odjadjare and Ekrakene (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 harsh condition. So proper treatment of milk, hygienic and clean environment of meat shop and poultry farm can reduce the contamination of *S. aureus* in the foods of animal origin.

The rapid emergence of antibiotic resistance in *S aureus* is the major cause of concern. The indiscriminate use of antibiotic agents for prophylactic and treatment purpose could be the reason for enhanced antibiotic resistance among *S. aureus*. Thus, there is possibility of the potential transmission of this drug resistant food borne pathogen (*S. aureus*) from animal to human through different foods of animal origin. The findings of the study also highlight the need for continuous surveillance of antibiotic susceptibility pattern of *S. aureus* isolated from foods of animal origin like milk, egg and meat. Also, to prevent the menace of antibiotic resistance, the prudent use of antibiotics to treat livestock diseases should be

done to overcome the problem of antibiotic resistance in future.

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