

Isolation, characterization and antibiotic susceptibility profile of *Staphylococcus aureus* from raw milk samples in Nagpur district, India

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Abstract: The study was carried out from July 2010 to June 2011. The aim of the present investigation was to isolate *Staphylococcus aureus* from raw milk obtained from cow from different parts of Nagpur district, India and to determine their antibiotic susceptibility profile. General biochemical tests along with the MASTASTAPH™ rapid agglutination tests were employed for bacterial identification. All the well characterized isolates were further confirmed by amplifying *coa* gene by PCR. A total 95 *S. aureus* isolates were obtained during this study from 50 raw milk samples. When all the well characterized isolates were subjected to antibiotic susceptibility testing, erythromycin and chloramphenicol were the most effective antibiotics. A large proportion of isolates were found to be resistant to Co-trimoxazole (89%) followed by Linomycin (81%) and Penicillin (35%) It was concluded from the current study that the hygiene of the milk was poor and resistant strains contaminated the milk probably during the process of transportation.

Keywords: Antibiotic susceptibility, *Staphylococcus aureus*, Raw milk

INTRODUCTION

Milk is a very nutritional food that is rich in carbohydrates, proteins, fats, vitamins and minerals. However, health risk to consumers can be associated with milk due to the presence of zoonotic pathogens and antimicrobial drug residues (Astal *et al.*, 2002). The quality of milk may be lowered by a number of factors such as adulteration, contamination during and after milking and the presence of udder infection (Bauer *et al.*, 1966). Pathogenic organisms in milk can be derived from the cow itself, the human hand or the environment (Astal *et al.*, 2002).

Staphylococci are normal Inhabitants of the skin and mucous membranes of animals and humans. Pathogenic strains are usually coagulase- positive (Braddy, 2002), and have been found to cause diseases in their hosts throughout the world (Chigbu and Ezeronye, 2003; Collins *et al.*, 2010). Diseases in cattle caused by *Staphylococcus aureus* range from simple abscesses and mastitis to the severe toxic shock syndrome (Devriese *et al.*, 1997; Chigbu and Ezeronye, 2003; Collins *et al.*, 2010).

Staphylococcal mastitis is the commonest and economically the greatest concern wherever dairy farming is practiced. The chief reservoir of this bacterium is an infected udder. A bacterium is shed into milk from infected quarters (Esrn *et al.*, 2005). The prevalence of mastitis and its associated pathogens in animals can be reduced by improved on the farm management techniques within the dairy industry (Evenson *et al.*, 1988). It is thus paramount importance to ensure that proper hygiene

practices are enforced in both the areas where the animals are kept and the milking environment.

Although it is difficult to control mastitis caused by *S. aureus* with antibiotics only (Farzana *et al.*, 2004), various antimicrobial agents are constantly being used to treat this disease in cattle (Garrod *et al.*, 1981). This practice results in the development of antibiotic resistant strains. The usage of antibiotics correlates with the emergence and maintenance of antibiotic resistant traits within pathogenic strains (Hassan *et al.*, 1978). These traits are coded by particular genes that may be carried on the bacterial chromosome, plasmids, transposones or on gene cassettes that are incorporated into integrons (Larsen *et al.*, 2000) thus are easily transferred among isolates.

Determination of levels of *S. aureus* and an evaluation of the antibiotic-resistant phenotypes of the isolates could serve as a tool for determining the hygiene standard implemented during milking. Data on antibiotic resistance could also be used to characterize these opportunistic pathogens, which may further limit the risks associated with the consumption of contaminated milk and its products (Mahon and Larsen, 1995).

The resistance of *S. aureus* isolates from different parts of the world to commonly used antibiotics has been widely reported (Odongo and Ambani, 1989; Matsunaga *et al.*, 1993; NCCLS, 1999; Malahat *et al.*, 2010). The study aimed at isolation and characterization of *S. aureus* by traditional and molecular biology techniques and determining the sensitivity pattern of different isolates of *S. aureus* from milk samples in Nagpur district, India

to commonly prescribed antibiotics.

MATERIALS AND METHODS

Place of work: The study was conducted at Department of Microbiology, Shivaji Science College, Nagpur India. Fifty milk samples were collected from different areas in Nagpur district during July 2010 to June 2011 and 95 strains were isolated from these milk samples.

Collection of samples: Fifty samples of raw milk (250ml) were collected aseptically in sterile glass bottles from milk vendors. All the milk samples were immediately transported and tested within three hours of their collection.

Isolation of presumptive *S. aureus* from milk samples: Two-fold serial dilutions were performed using 2% peptone water and aliquots of 100µl from each dilution were spread plated onto Manitol salt agar (MSA) Himedia- Mumbai. The plates were incubated aerobically at 37°C for 18hrs- 24hrs. Consequently the 20 characteristic *S. aureus* colonies those were yellow in color from each MSA plate were further purified by sub culturing onto MSA plates and the plates were retained for further bacterial identification.

Bacterial identification: Gram staining was performed and Gram positive cocci that occurred in clusters under the microscope were subjected to preliminary biochemical tests (catalase and oxidase tests). The identities of the isolates were confirmed based on positive results for the DNase test, beta hemolytic patterns on blood agar enriched with 5% (v/v) blood and the rapid latex agglutination slide test for *S. aureus* using the MASTASTAPH™ (Onasanya *et al.*, 2003). The slide agglutination test was performed according to the manufacturer's instructions.

In addition to the above characterization all the previously characterized *S. aureus* isolates were further confirmed by amplifying *coa* gene (Pitkala *et al.*, 2004).

Antibiotic susceptibility testing: Antibiotic susceptibility test was performed on all the *S. aureus* isolates to determine their antibiotic resistance profile. Overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100iL) from each isolate suspension was spread plated on Muller Hinton agar (MHA) (Himedia, India). Susceptibilities of the isolates to a panel of 13 different antibiotic discs were determined. The antibiotics tested are shown in fig. 1 and are selected because large numbers of bacteria resistant to these had been documented in the study area. Antibiotic discs were gently pressed onto the inoculated Muller Hinton agar to ensure intimate contact with the surface and the plates were incubated overnight (Sears, 2003). Inhibition zone diameters were measured and values obtained from the National Committee on Clinical Laboratory Standards were used to interpret the results obtained. *S. aureus*

isolates were classified as resistant, moderately sensitive or susceptible to a particular antibiotic.

RESULTS

In a total of 50 raw milk samples, *S. aureus* was biochemically, serologically and genetically identified in 95 isolates. These 95 isolates were subjected to thirteen commonly prescribed antibiotics (Fig.1).

According to the results of antibiotic sensitivity of *S. aureus*, 100% susceptibility was observed against erythromycin and chloramphenicol. Gentamycin was found to be sensitive against 96% *S. aureus* isolates. The susceptibility of the other antibiotics in decreasing order against *S. aureus* was found to be Kanamycin (92%), Streptomycin (91%), Methicillin (89%), Ampiclox (86%) and Augmentin (81%). A large proportion of isolates were found to be resistant to Co-trimoxazole (89%) followed by linomycin (81%), Penicillin (35%) and Ampicillin (32%).

DISCUSSION

In the current study, we described the isolation and antibiotic susceptibility characterization of *S. aureus* from raw milk obtained from different areas of Nagpur district, India. The present study pointed out that the milk drawn from healthy animals may be free of bacteria but it becomes contaminated by hands of milkman or from the udders of animals harboring microorganisms. Dirty teats with dung and mud are the dirt source of bacteria from milk. Moreover the utensils used for the milk are also the source of various types of bacteria but the main source is the contaminated water that is added to milk to increase its quantity. All these results showed that raw milk passes through very unhygienic condition during transportation. Moreover, it takes long time to reach the consumer and during that time it becomes highly contaminated because of high temperature which causes the proliferation of bacteria. Hassan *et al.* (1978) studied tetracycline and erythromycin as effective antibiotics against *S. aureus*. Shoemaker and Yow (1954) and Uwaezuoke and Aririatu (2004) also reported similar findings against *S. aureus* isolates when large doses of erythromycin were given intravenously. The results of the present study are similar to those of above workers. In addition to this in given study Chloramphenicol was also found to be effective against all the isolates of *S. aureus*. According to Garrod *et al.* (1981), Penicillin is still effective antibiotic against *S. aureus*. In the study 65% isolates of *S. aureus* were sensitive to penicillin and more than 30% were resistant, this may be due to extensive use of penicillin that has developed resistance. Devriese *et al.* (1997) isolated *S. aureus* strains from bovine mastitis and reported that β -lactamase labile penicillin's had 51% isolates resistant in 1996, tetracycline resistant strains were 21% in 1971 and 9% were found during 1996 and 10% resistant isolates

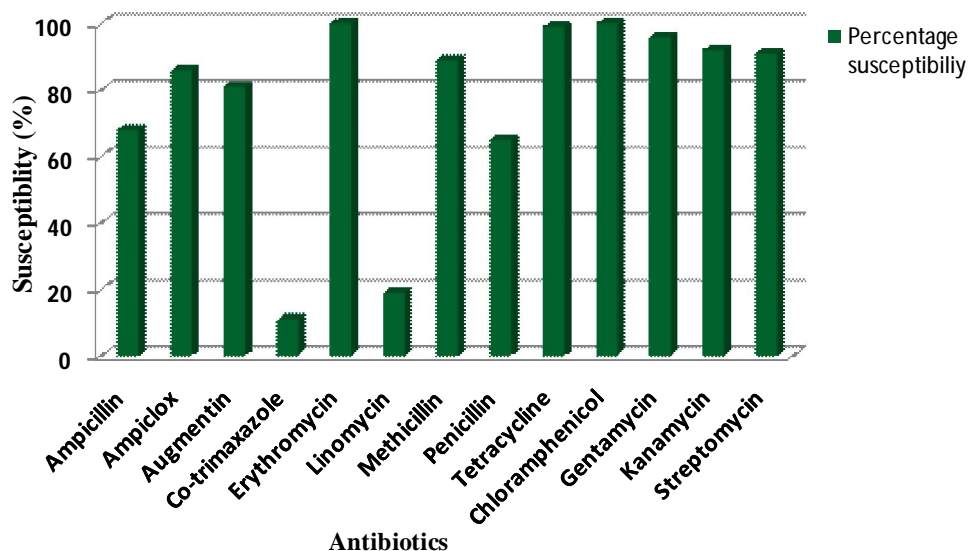


Fig.1. Susceptibility pattern of 95 isolates of *S. aureus*.

with linomycin in 1996. The results of the present study were similar to the work carried out by Devriese *et al.* (1997).

In the present work more than 86% isolates were sensitive and 12% isolates were resistant to ampiclox. Augmentin showed 81% sensitivity against *S. aureus*. In case of Co-trimoxazole 89% isolates of *S. aureus* were found resistant to these antibiotics, this may be due to the excessive and long term use of antibiotics.

The present study demonstrated that the resistant strains may have been transferred to cow and then to milk, which can be the reason of infection in human beings if we take raw milk. This can be prevented by improving hygienic condition and careful handling of cow during milking.

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