Prevalence and Antimicrobial Susceptibility of Drug Resistant *Staphylococcus aureus* in Raw Milk of Dairy Cattle

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The sturdy was carry out to evaluate the incidence and of multidrug resistant *Staphylococcus aureus* in raw milk of dairy cattle. Isolation and identification of *S. aureus* were performed from 4 types of cattle raw milk samples. The isolates were tested using Kirby-Bauer disk diffusion method for their antimicrobial susceptibility to 15 different antimicrobial drugs. A total of 115 milk samples from cattle were cultured for incidence of *S. aureus,* and 34.78% of the samples tested were from fresh cow milk, 34.78% from buffalo milk, 17.39% from goat milk and 13.04% from sheep milk samples. *S. aureus was* isolated from a total of 25 (21.73%) of the 115 samples. Almost 80-90% of the isolates were showed multiple drug resistance to majority of the antimicrobial agents tested. Several isolates from milk samples were found resistant to Nalidixic acid, Amoxycillin+sulbactam, Cloxacillin, Erythromycin, Kanamycin and Vancomycin. On the other hand several isolates were found susceptible to the Ofloxacin, Ampicillin, Tetracycline Oxacillin, Streptomycin, Sulphafurazole and Ciprofloxacin. The present study provides preliminary information on incidence of antibiotic resistant *S. aureus* as milk contaminants which may act as vehicles for the transmission of drug resistance in food.

Keywords: Antimicrobial drug resistance, *S. aureus,* dairy cattle, mastitis, food infections and Food handling.

INTRODUCTION

There is a growing market for locally produced dairy products in India. Farm-dairies commonly use raw milk for cheese and other locally produced preparation, rely on locally available water supply, availability of food and feed close to the dairy facilities. These factors may increase the risk for contamination of dairy products with pathogenic bacteria (Oliver et al., 2005). *Staphylococcus aureus* is a versatile pathogen of humans and animals causes a wide variety of diseases from mild skin infection to more severe diseases such as pneumonia and septicaemia (Lowy, 1998). *S. aureus* is present on the skin and mucosae of food producing animal, such as ruminants and it is commonly associated to mastitis leading to contamination of milk and dairy products (Jablonsky and Bohach, 1997). *S. aureus* is a human pathogen may cause several infections (Lowy, 1998) and bacteraemia (Reacher et al., 2000). In the last few decades Staphylococcal food poisoning has been reported as third cause of food-borne illnesses in the world (Zhang et al., 1998). Among the foods implicated in SFP, milk, dairy products and meats, particularly handled foods, play an vital function since enterotoxigenic strains of *S. aureus* have been commonly isolated in them (De Buyser et al., 2001). Antimicrobial resistance is a main public health worry worldwide. The expansion of resistance both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals (McNamara, 2000). Several authors observed that administration of antibiotics to food-producing animals for remedial purposes or as growth promoters may be a primary factor in selecting for antimicrobial-resistant bacteria (Barber et al., 2003).

The source of milk contamination may be due to environment, milking utensils and the personnels. The pathogenic agents, including the staphylococci, occurring

Table 1. Incidence of *Staphylococcus aureus* in raw milk.

Source	No of Isolates	% of Isolates	Number of sample positive for *S.aureus*
Cow milk	40	34.78	11
Buffalo milk	40	34.78	8
Goat milk	20	17.39	4
Sheep milk	15	13.04	2
Total	115		25

in milk may be either a cow, or a human and it may be transmitted by both. The aims of present work were: i) to evaluate the occurrence of *S. aureus* in milk, dairy products ii); morphological, biochemical and serological identification of isolates iii) Antimicrobial profiling of the isolated strains based on their antimicrobial-resistance pattern.

MATERIALS AND METHODS

Sample collection

A total of 115 (40 fresh Cow milk samples, 40 buffalo fresh milk samples, 20 fresh goat milk samples and 15 fresh sheep milk samples) samples were collected from various places in Meerut region and animal husbandry department dairy farms of babugarh cantt (India) from June 2009-May 2010 (as described in Table 1). Properly packed samples were placed on ice or frozen refrigerant packs in an insulated box, and then stored in laboratory at 4°C for further processing and study.

Microbiological examinations

Isolation and identification of *S.aureus* were performed according to the National Mastitis Council recommendations. Immediately after delivery, the raw milk samples were inoculated on Mannitol salt Agar plates (Himedia, India). Samples were incubated for 24 to 48 h at 37^0C and observed for bacterial growth. Bacterial colonies subcultured on the same selective media plates and incubated at 37°C for 24 hrs in order to obtain pure cultures. The purified colonies were subsequently preserved in Nutrient agar slants and also preserved in 50% glycerol stock solution for the further use.

Identification of bacterial cultures under microscope

The purified cultures of bacteria isolates obtained on Mannitol salt agar media plates were further examined under the microscope for their morphological characters (convex elevation and smooth margins staining, and cultural characteristics) and gram staining.

Biochemical Identification with commercial kit based methods (Himedia, India)

The biochemical tests were performed with Himedia Identification kit which includes Voges-Proskauer reactions, phosphotase, ONPG, Urease production, arginine utilization, and 7 different carbohydrates utilization tests- Mannitol, Sucrose, Lactose, Arabinose, Raffinose, Trehalose and Maltose. Further identification of isolates was examined with the standard chart (Supplied by Himedia, India).Catalase test was performed according to a tube method using 3% Hydrogen peroxide. Appearance of bubbles confirmed presence of enzyme catalase.

Serological Identification

Further the isolates were observed for hemolysis and coagulase production. Coagulase test was performed according to a tube method using rabbit plasma in 1:10 dilution in nutrient broth (Arbiet, 1988). Further DNase test (Weckman and Catlin, 1957) was performed to detect the degradation of deoxyribonucleic acid (DNA) on DNase test agar (Himedia). The depolymerization of the DNA was detected by flooding the surface of the medium with 1 N HCl and observing for clear zones in the medium surrounding growth. A positive reaction, pink to red zone around the growth was observed.

Antibiotic susceptibility testing (In accordance to NCCLS)

The isolates were tested for their susceptibility to 15 different antimicrobials drugs (Himedia, India) using Kirby-bauer disk diffusion method: Ofloxacin (5 mcg), Ampicillin (25 mcg), Tetracycline (30 mcg), Kanamycin (30 mcg), Erythromycin (10 mcg), Azithromycin (15 mcg), Nalidixic acid (30 mcg), Amoxycillin+ Sulbactam (30/15 mcg), Streptomycin (25 mcg), Sulphafurazole (25 mcg), Ciprofloxacin (30 mcg), Rifampicin (30 mcg), Vancomycin (5mcg), Oxacillin (5mcg), Cloxacillin (10mcg) were used. By the standard method of inoculation, a loopful culture of isolates was inoculated into 2 ml Mueller-Hinton broth. The broth culture was incubated at 37°C for 4 hours to

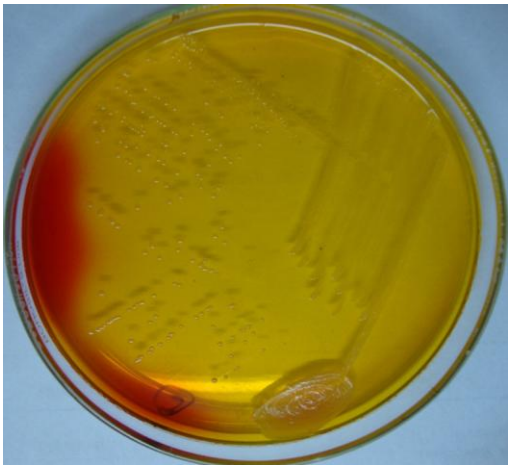

Figure 1. Mannitol fermentation by *S. aureus*.

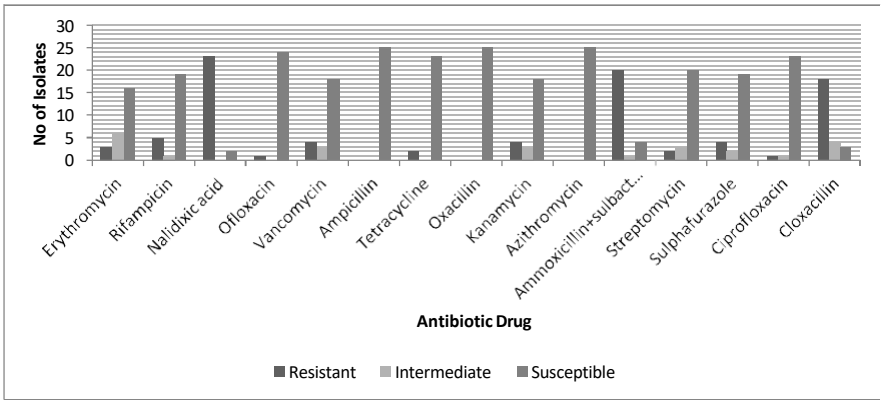

Figure 2. Susceptibility of *Staphylococcus aureus* against various Antibiotics.

obtain fresh culture.

The concentration of actively growing cultures was then adjusted to a 0.5 McFarland standard. A sterile cotton swab was dipped into broth suspension within 15 minutes and excess broth purged by pressing and rotating the swab firmly against the inside of the tube. The swab was then spreaded evenly over the entire surface of the plate agars to obtain uniform inoculums. Plates were then allowed to dry for 3 to 5 minutes. The antibiotic disks were gently applied to ensure their contact with the inoculated Mueller-Hinton agar surface, and incubated at 37^0C. The plates were observed after 18-24 h and the zones of inhibition were measured by antibiotic susceptibility scale (Himedia, India). The zone diameter for individual antimicrobial agents was then translated into susceptible and resistant categories according to the interpretation table (supplied by the Himedia, India).

RESULTS

A total of 115 milk samples from various sources were cultured for incidence of *S. aureus*

Biochemical identification

From all 115 samples, 25 samples showed growth on Mannitol salt agar (Figure 1). After biochemical characterization which involves, coagulase, and catalase, identified by rapid identification kits (Himedia, India), only 25 samples showed positive result for all the Morphological, biochemical and serological tests. Positive samples for *S. aureus* included 11 cow milks, 8 buffalo milks, 4 goat milks and 2 sheep milks.

Antimicrobial susceptibility

Almost 60-70% of the isolates were observed with multiple drug resistance to majority of the antimicrobial agents tested (Figure 2). Several isolates from milk samples were found resistant to Nalidixic acid, Amoxycillin+sulbactum, Cloxacillin, Erythromycin,

Kanamycin and Vancomycin. On the other hand several isolates were found susceptible to the Ofloxacin, Ampicillin, Tetracycline Oxacillin, Streptomycin, Sulphafurazole and Ciprofloxacin. Various isolates also exhibited intermediate resistance to Erythromycin, Vancomycin, Streptomycin and Cloxacillin.

DISCUSSION

In this study we describe the isolation, identification and antibiotic susceptibility characterization of *S. aureus* from milk obtained from different dairy cattle. Our results indicate that 25 samples were positive for *S. aureus.* Various studies have been conducted to evaluate the prevalence of *S. aureus* in milk obtained from communal and commercial farms. The results reported in our study are likewise high when compared to those formerly documented (Shitandi and Sternesjö, 2004; Gündoğan et al. 2006). Based on observations made throughout the collection of samples, we therefore report that improper hygiene and poor farm management practices contributed to the presence of *S. aureus* in the milk, especially in those from the communal farms. The *S. aureus* incidence at a considerable high percentage indicates the alarming situation both for dairy farming and for public health as well. The numerous examples of *S. aurues* causing bacteremia were reported in human with predisposing conditions of dairy farms (Normanno et al., 2007). The presence of *S. aurues* in the milk sample is a new and appealing as well as an important finding of this study. *S. aureus* was resistant to multiple classes of antibiotics which can cause serious health problems (Tenover, 2006). In the present study 115 raw milk samples were screened for the incidence of *S. aureus* isolates exhibited multiple drug resistant. A total of 25 raw milk samples were found positive for the presence of *S. aureus*. Several *S. aureus* isolates from milk samples were found resistant to Nalidixic acid (Kresken and Wiedemann, 1988), Amoxycillin+sulbactam (Liu and Lewis, 1992), Cloxacillin (Akbarzadeh et al., 2010), Erythromycin (Linda et al., 2010), Kanamycin (Virdis et al., 2010) and Vancomycin. On the other hand several isolates were found susceptible to the Ofloxacin, Ampicillin, Tetracycline, Oxacillin, Streptomycin, Sulphafurazole and Ciprofloxacin. In the present study several strain were observed with multiple drug resistance. But several isolates were found susceptible for Ofloxacin, Ampicillin, Tetracycline, Oxacillin, Streptomycin, and Ciprofloxacin. These susceptible antibiotic drugs will be used as the effective drugs against staphylococcal infections. The present study demonstrated that the resistant strains may be transferred to milk from infected udders, poor farm practices and due to poor handling during milking, it transmitted to the milk utensils, which can be the reason of infection in human beings. The over dose of antibiotics use during farm practices are also responsible for the emergence of antibiotic resistant microorganisms. Regular Health checkups of dairy cattle, sterilization of dairy equipment, washing of utensils, milking workers hands, udders, pasteurization/boiling of milk should be practiced.

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