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ANTIBIOTIC RESISTANCE PATTERN AGAINST VARIOUS ISOLATES OF *STAPHYLOCOCCUS AUREUS* FROM RAW MILK SAMPLES

Kalsoom Farzana[1], Syed Nisar Hussain Shah[1] and Farzana Jabeen[2]
[1]Department of Pharmacy, Bahauddin Zakariya University, Multan.
[2]Wilson Pharmaceutical, Islamabad, Pakistan.

Abstract: A total of 50 raw milk samples were taken from shops in Multan city. More than 40% milk samples were found contaminated more than 10^8 bacteria per ml. The coagulase-positive *Staphylococcus aureus* was present in all the samples. When the coagulase-positive *Staphylococcus aureus* isolates were subjected to antibiotic sensitivity test, the erythromycin was the most effective antibiotic. Only 10% isolates were found methicillin resistant, co-trimaxazole, showed maximum resistance rate 81.81%, 57% isolates were resistant to lincomycin, 63.63% to orbenin, 25.97% to ampicillin, 24.67% to penicillin 23.37% to augmentin and 18.18% to ampiclox. Only 2.6% of the isolates were found resistant to tetracycline. It is concluded that hygiene of milk is poor and resistant strains have contaminated the milk probably during the process of transportation.

Keywords: Antibiotic resistance, isolates, raw milk, *Staphylococcus aureus.*

INTRODUCTION

Milk is an excellent bacteriological medium for a large number of micro-organisms. When the milk is drawn from the udder of a healthy animal, it contains organisms that have entered the teat canal through its opening. They are mechanically flushed out during milking. The number ranged during milking between several hundreds to several thousands per millilitre. The source of contamination may be due to environment, milking utensils and the personals. *Staphylococcus aureus* isolates are normal inhabitants of skin and mucus membranes. The coagulase-positive staphylococci constitute the well known pathogenic species *Staphylococcus aureus* [Mahon and Larsen 1995]. A variety of diseases may be potentially transmitted through milk. The source of pathogenic agents occurring in milk may be either a cow, or a human, and it may be transmitted by both [Seguin *et al.* 1999, Khan *et al.* 2000]. The most important microorganisms causing mastitis are Staphylococci, Streptococci and Coliform bacteria. The different diagnosis of clinical mastitis can be treated with various antibiotics [Morin *et al*. 1998].

Antibiotics are used to treat diseases of cattle and as well as used as preservatives for milk [Devriese *et al.* 1997]. The indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective [Johnston *et al.* 1983]. Resistant bacteria occur in soil, water, plants and animals. The resistant bacteria present in environments are in contact with human beings and animals. It has been estimated that nearly equal tonnage of antimicrobial agents are used in man and in agriculture worldwide [EFA 1997]. When low doses of antibiotics are used, they inhibit the growth of

susceptible bacteria while resistance bacteria thrive and grow such as in the presence of tetracycline [Eichner and Gravitz 1999].

The present article reports the antibiotic resistance of the *S. aureus* in raw milk samples collected from various shops in Multan city.

MATERIALS AND METHODS

The study was conducted at the Microbiology Laboratory, Department of Pharmacy, Bahauddin Zakariya University Multan. Fifty milk samples were collected from different areas in Multan during January 1992 to December 1992 and seventy seven strains were isolated from these milk samples.

COLLECTION OF SAMPLES

Fifty samples of raw milk (250ml) were collected aseptically in sterilized glass bottles from milk vendors. All milk samples were immediately transported and tested within three hours of their collection. The serial decimal dilutions of these milk samples were then prepared in 0.1% peptone, which was supplemented with 0.05% (w/v) of Tween-80 and 0.1% (w/v) Mg Cl_2. $6H_2O$ [Lachica 1984].

These dilutions were prepared in duplication and then transferred to Plate Count Agar (PCA), and Baird Parker Agar (BPA). All plates were incubated at 37^{o}C for 48 hours. PCA and BPA were supplemented with 0.05 Tween-80 and 0.1% $MgCl_2.6H_2O$ for resuscitation of stressed bacterial cells [Lachica 1976, 1984].

MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

Identification, morphological and biochemical characterization of bacterial strains were tested in the Laboratory. The presence of colonies was confirmed by the following tests were carried out:

Haemolysis	[Pelczar *et al*. 1999]
Coagulase	[Pelczar *et al*. 1999]
DNase	[Collins *et al*. 1995]
Catalase	[Pelczar *et al*. 1999]

DISC DIFFUSION (BAUER-KIRBY) SUSCEPTIBILITY TEST

The disc diffusion test was done for each isolate on Mueller-Hinton agar (CM337-OXOID), 25 ml of medium was poured into 90 mm diameter sterile Petri dishes to a depth of 4 mm on a level surface to make the depth of the medium uniform and left at 37^{o}C temperature overnight to check sterility [Bauer *et al*. 1966].

For inoculum's preparation 5 ml tryptic soya broth (CM129-OXOID) was dispensed in screw capped tubes and sterilized by autoclaving at 121°C for 15 minutes. The tubes were cooled and kept in an incubator for 24 hours at 35°C to check sterility. The each isolate was inoculated in the

sterilized tubes containing the medium, and placed in an incubator overnight at 35°C. The presence of turbidity in broth cultures was adjusted according to 0.5 McFarland standards by the addition of sterilized plain broth [NCCLS 1993].

PREPARATION OF INOCULUM FOR DISC DIFFUSION TEST

Standardized bacterial suspension was saturated with a sterile Dacron tip swab and excess culture was removed by turning the swab against the side of the tube. Inoculum was spread evenly over the entire surface of the Mueller-Hinton agar plates by swabbing back and forth across the agar in three directions to give a uniform inoculum to the entire surface. These plates were allowed to dry before applying discs and after 15 minutes discs of given potency were applied on the inoculated plates with the help of forceps. These plates were then placed in an incubator at 35°C for 18 hours in inverted position. After 18 hours of incubation, plates were examined and the zone of inhibition was measured. All gram positive cocci were also assessed for the oxacillin and methicillin resistance by the same procedure as described above.

RESULTS

In a total of 50 raw milk samples, *S. aureus* was biochemically identified in 77 isolates. These 77 isolates were subjected to ten antibiotic sensitivity tests (Table 1). Raw milk samples were found to be excessively contaminated as all the samples contained more than 10^8 microorganisms per ml of milk and indicated poor hygienic quality of milk.

Table 1: Antibiotic sensitivity of *S. aureus* from milk samples.

Antibiotics used	Percentage susceptibility of seventy seven isolates		
	Resistant	Moderately susceptibility	Susceptible
Methicillin	8 (10.39%)	7 (9.09%)	62 (80.52%)
Ampicillin	20 (25.97%)	12 (15.58%)	45 (58.44%)
Tetracycline	2 (2.60%)	21 (27.27%)	54 (70.13%)
Ampiclox	14 (18.18%)	3 (3.89%)	60 (77.92%)
Erythromycin	2 (2.60%)	1 (1.3%)	74 (96.10%)
Lincomycin	44 (57.17%)	19 (24.67%)	14 (18.18%)
Co-trimaxazole	63 (81.81%)	8 (10.39%)	6 (7.79%)
Penicillin	19 (24.67%)	13 (16.88%)	45 (58.44%)
Augmentin	18 (23.37%)	0 (0%)	59 (76.62%)
Orbenin	49 (63.63%)	0 (0%)	28 (36.36%)

According to the results of antibiotic sensitivity of *Staphylococcus aureus*, 96.10% organisms were susceptible to erythromycin. Only 1.3% was moderately susceptible and 2.60% resistant to erythromycin. The susceptibility of the other antibiotics in decreasing order against *Staphylococcus aureus* was found to be methicillin (80.52%), ampiclox

(77.92%), augmentin (76.62%), tetracycline (70.13%), ampicillin and penicillin (58.44%), orbenin (36.36%), lincomycin (18.18%) and co-trimaxazole (7.79%).

Whereas resistance pattern of the co-trimaxazole, orbenin, lincomycin, ampicillin, penicillin, augmentin, ampiclox, methicillin, tetracycline and erythromycin was in decreasing order 81.81%, 63.63%, 57.17%, 25.97%, 24.67%, 23.37%, 18.18/%, 10.39%, 2.60% and 2.60% isolates respectively (Table 1 and Fig. 1).

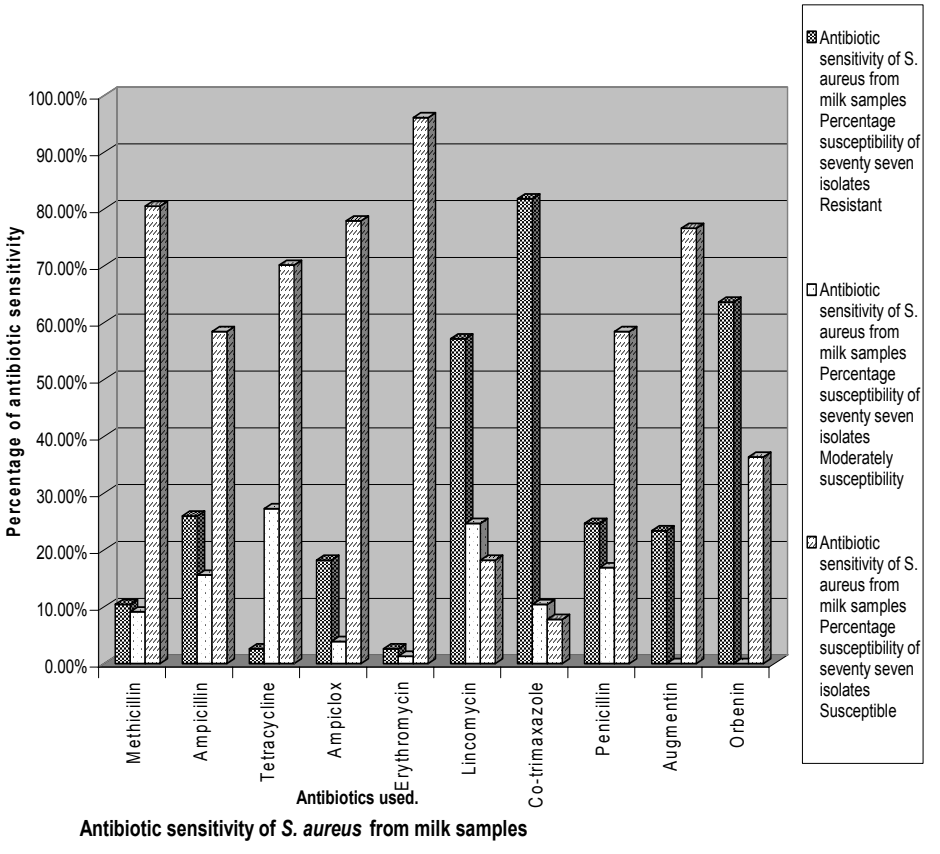

Fig. 1: Antibiotic sensitivity of *S. aureus* isolated from milk samples.

DISCUSSION

The present study pointed out that the milk drawn from healthy animals may be free of bacteria but it becomes contaminated by hands of milk man or from the udders of animals harboring microorganisms like Streptococci, Staphylococci, Corynebacteria, Coliforms, Klebsiellae, Salmonellae and others. Dirty teats with dung and mud are the dirt source of bacteria for milk. Moreover, the utensils used for 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 conditions 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.

ANTIBACTERIAL ACTIVITY

Hassan *et al.* [1978] studied erythromycin and tetracycline as effective antibiotics against *Staphylococcus aureus.* Shoemaker and Yow [1954] also reported similar findings against *Staphylococcus aureus* isolates when large doses of erythromycin were given intravenously. The results of the present study are similar to those of above workers.

According to Garrod and co-workers [1981] penicillin is still effective antibiotic against *Staphylococcus aureus*. In the study 58% isolates of *Staphylococcus aureus* were sensitive to penicillin and more than 24% resistant, probably this may be due to the excessive rational or irrational use of the penicillin that has developed resistance. *Staphylococcus aureus* isolates from mastitis when treated with penicillin, tetracycline, lincomycin, 81% isolates were resistant to penicillin during 1977, 3% resistant to tetracycline and 10% resistant to lincomycin in the year 1996 [Devriese *et al*. 1997].

Devriese *et al.* [1997] isolated *S. aureus* strains from bovine mastitis and reported that Beta-lactamase-labile penicillins had 51% isolates resistant in 1996, tetracyclines resistant strains were 21% in 1971 and 9% were found during 1996 and 10% resistant isolates with lincomycin in 1996. The results of present study were quite similar to Devriese *et al.* [1997] study as methicillin had 10% resistant isolates to methicillin in 1977 but the results of ampicillin and penicillin were having lesser resistant rate 25.97%, 24.67% and of high resistant rate with lincomycin 57.14% in the study.

In present work more than 77% isolates were sensitive and 18.18% isolates were resistant to ampiclox. Augmentin is a combination of amoxicillin and clavulanic acid, 23.37% isolates was found to be resistant to augmentin. In case of orbenin, 63% isolates were found to be resistant, may be due to the excessive use of this antibiotic. In case of co-trimaxazole 81.81% isolates of *Staphylococcus aureus* were found resistant to these antibiotics this may be due to the excessive and long term use of the antibiotic.

Moreover, when low doses of antibiotics were used against bacteria, they inhibit the growth of susceptible bacteria and leave smaller number of already resistant bacteria, which thrive and grow. These bacteria spread their resistance traits to other previously non-resistant cells than eventually affecting other cells [Estes 1998, Craig 1998, McGowan and Bowker 1998, Lacy *et al.* 1998, Eichner and Gravitz 1999].

The present study demonstrated that the resistant strains may have been transferred to cow then to milk, which can be the reason of infection in

human beings if we take raw milk. These can be treated by improving hygienic conditions and careful handling of cow during milking.

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