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Sensitivity Pattern of *Staphylococcus aureus* Isolates from Different Sources for Methicillin, Vancomycin, β-lactamase and ESBL Production

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ABSTRACT

Background: *Staphylococcus aureus* has the ability to develop many efficient mechanisms to neutralize them and it has become difficult to control the virulent strains of *S. aureus* from causing staphylococcal diseases in animals and humans. Mostly Staphylococcal strains have become resistant to methicillin, β-lactamase and ESBL activity and sometime to vancomycin also. The present study investigated the phenotypic and genotypic characteristics of all the 62 *S. aureus* isolates for sensitivity towards methicillin, β-lactamase, ESBL production and vancomycin.

Methods: The isolates were obtained by conventional microbiological methods, confirmed genotypically by 23S rRNA ribotyping and Maldi-Tof MS. Methicillin resistance activity among *S. aureus* isolates was detected by culturing them on MeReSa Agar. Extended-spectrum β-lactamase activity among *S. aureus* isolates was detected by the combined disc method.

Result: On MeReSa agar, 53(85.48%) isolates were detected as methicillin resistant *S. aureus* (MRSA), but none of the isolates from any source or place of sampling was detected positive by the methicillin disk method. Extended-spectrum β-lactamase (ESBL) activity was exhibited by 51 (82.25%) isolates with 100% (maximum) isolates from human pus showing activity and 66.66% (least activity) was seen in isolates from unprocessed meat. All the isolates were susceptible to vancomycin.

Key words: ESBL activity, MRSA, *Staphylococcus aureus,* VRSA, β-lactamase.

INTRODUCTION

Over the past few years, it has become hard to control the virulent strains of *S. aureus* because they have become resistant to various β-lactam antibiotics such as methicillin and penicillin. Such strains of *S. aureus* are known as methicillin-resistant *S. aureus* (MRSA). Methicillin-resistant *S. aureus* was reported in October 1960, which is now endemic in India (Ray *et al.,* 2013). The incidence of MRSA varies from 25% in the western part of India (Patel *et al.,* 2010) to 50 percent in South India (Gopalkrishan *et al.,* 2010). Since then, MRSA has become endemic in hospitals and nursing homes worldwide.Beta-lactam compounds such as penicillin continue to be one of the most frequently used drugs in veterinary medicine (Pitkala *et al.,* 2007). With the development of MRSA, vancomycin has been used as the antibiotic of choice to treat infections caused by *S. aureus* strains that are resistant to methicillin and oxacillin. In addition, the emergence of vancomycin-resistant *S. aureus* has been reported in some studies (Tenover *et al.,* 2004; Ateba *et al.,* 2010). The establishment of MRSA and the emergence of VRSA have great concern because these are not only resistant to methicillin but also to vancomycin, monobactams and cephalosporins through the production of ESBL (Extended-spectrum β-lactamases). Production of other Extended-spectrum β-lactamase (ESBL) enzymes leads resistance to penicillin and cephalosporins, monobactams and carbapenems antibiotics. Resistance mechanisms of staphylococci include enzymatic inactivation of the antibiotic (penicillinase and aminoglycoside-modification enzymes), alteration of the target with decreased affinity for the antibiotic (penicillin-binding protein 2a of methicillin-resistant *S. aureus* and D-Ala-D-Lac of peptidoglycan precursors of vancomycin-resistant strains), trapping of the antibiotic (vancomycin and daptomycin) and efflux pumps (fluoroquinolones and tetracycline) (Lowy, 2003; Pantosti *et al.,* 2007). MRSA and VRSA strains show pathogenic and epidemiological characteristics in several

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ways, such as clonal evolution (Fitzgerald *et al.,* 2001), mutation and horizontal gene transfer (Brody *et al.,* 2008). Although there are many reasons which compromise antibiotic treatment of *S. aureus* infections of which resistance activity of bacteria toward antibiotics is the most important. The present study investigated the phenotypic and genotypic characteristics of all the 62 *S. aureus* isolates with respect to MRSA, β-lactamase, ESBL production and VRSA activities.

MATERIALS AND METHODS

Sample collection

A total of 180 samples from various sources like human pus, animal pus, the skin of an animal, skin of human, mastitis milk, regular milk and unprocessed meat were collected. These sample sources belonged to two different localities of Bikaner (Rajasthan). The samples were collected in the morning and immediately taken to the laboratory on ice for further processing.

Isolation and identification of bacteria

The organisms were isolated and identified as described by Cowan and Steel (2003) and Quinn *et al.* (1994). Each sample was swabbed on nutrient agar medium and then incubated overnight at 37°C. Suspected colonies were streaked on mannitol salt agar in primary, secondary and tertiary fashion and incubated for 24 h at 37°C. Of the 180 samples, 62 isolates of *S.aureus* from various sources were obtained, further confirmed genotypically by 23S rRNA-based ribotyping and Proteomic based identification of *S.aureus* by MALDI TOF MS (VITEK MS RUO).

Methicillin resistance activity

Methicillin resistance activity among *S. aureus* isolates was detected by culturing them on MeReSa Agar. This method included observation of colony growth on MeReSa agar base with MeReSa selective supplement (FD229) and Cefoxitin supplement (FD259). After inoculation of testing isolate, the methicillin positive strain grew as luxuriant greenish pink color colony after incubation at 35-37°C for 18-48 hours (Alwash and Saleh, 2013).

Beta-lactamase activity (Acidimetric method)

Hydrolysis of the β-lactam ring generates a carboxyl group, acidifying un-buffered systems. The resulting acidity can be tested in tubes. The technique described by Livermore and Brown (2001) in which 2 ml of 0.5% (w/v) aqueous phenol red solution was diluted with 16.6ml distilled water and 1.2 g of benzylpenicillin was added. The pH was adjusted to 8.5 with 1M NaOH. The resulting solution, violet in color, was stored at -20°C. Before use, 100 µl of the solution was distributed into microtitre wells and inoculated with the bacterial isolates to produce dense suspensions. A yellow color within 5 min indicated β-lactamase activity.

Extended-spectrum β-lactamase (ESBL) activity

Extended-spectrum β-lactamase activity among *S. aureus* isolates was detected by the combined disc method described by Livermore and Brown (2001). This Method included comparing the zone given by discs containing an extended-spectrum cephalosporin with and without clavulanic acid. If an ESBL is produced, the zones are increased ≥5 mm for the discs containing clavulanic acid. This method recommends comparison of the zone given by cefotaxime 30 µg versus cefotaxime 30+Clavulanic acid 10 µg and ceftazidime 30 µg versus ceftazidime 30 +clavulanic acid 10 µg. The readymade (HiMedia) discs were used for this test as described.

RESULTS AND DISCUSSION

Staphylococcus aureus isolation and genotypic confirmation

On the basis of cultural and biochemical properties, out of 180 samples, 62 isolates, including 8 from human pus samples, 9 from animal pus, 12 from mastitis milk, 10 from normal milk, 7 from human skin, 7 from animal skin and 9 from unprocessed meat, were presumptively identified as *S. aureus* (Table 1). These 62 isolates were genotypically confirmed as *S. aureus* by 23S rRNA ribotyping using the following sequence for the Primer F-5′-ACG GAG TTA CAA AGG ACG AC-3′ and R-5′-AGC TCA GCC TTA ACG AGT AC-3′, producing an amplicon of 1250 bp (Fig 1) and proteomically confirmed by Maldi-tof MS. An overall recovery rate was 34.44%. These results are almost similar to those reported by Yadev *et al.* (2015).

Methicillin, β-lactamase, ESBL and vancomycin activity

Of the 62 isolates, phenotypically, 53(85.48%) were detected as methicillin resistant *S. aureus* (MRSA) on MeReSa agar base (MeReSa Selective Supplement having Methicillin

Table 1: Sensitivity of *S. aureus for* methicillin, vancomycin, β-lactamase production and ESBL activity.

Source of isolates	MeReSa activity		β-lactamase production		ESBL production		Vancomycin sensitivity	
	P	N	P	N	P	N	P	N
Mastitis milk (12)	10 (83.33%)	2 (16.66%)	9 (75%)	3 (25%)	11 (91.66%)	1 (8.33%)	0 (0%)	12 (100%)
Normal milk (10)	9 (90%)	1(10%)	8 (80%)	2 (20%)	8 (80%)	2(20%)	0 (0%)	10 (100%)
Pus of animal (9)	9 (100%)	0 (0%)	7 (77.77%)	2 (22.22%)	7 (77.77%)	2 (22.22%)	0 (0%)	9 (100%)
Pus of human (8)	7 (87.5%)	1 (12.5%)	6 (75%)	2 (25%)	8 (100%)	0 (0%)	0 (0%)	8 (100%)
Skin of animal (7)	5 (71.42%)	2 (28.57%)	6 (85.71%)	1 (14.28%)	5 (71.42%)	2 (28.57%)	0 (0%)	7 (100%)
Skin of human (7)	7 (100%)	0 (0%)	5 (71.42%)	2 (28.57%)	6 (85.71%)	1 (14.28%)	0 (0%)	7 (100%)
Unprocessed meat (9)	6 (66.66%)	3 (33.33%)	7(77.77%)	2 (22.22%)	6 (66.66%)	3 (33.33%)	0 (0%)	9 (100%)
Total (62)	53	9	48	14	51	11	0	62

P= Positive, N= Negative.

2 mg/ml+ cefoxitin 3 mg/ml in 100 ml media) (Fig 2) but none of the isolates from any source or place of sampling was detected positive by methicillin disk (5 mcg) method (Table 1). Phenotypically 100% MRSA isolates from animal pus and human skin were detected by MeReSa agar. While least number (66.66%) isolates from unprocessed meat were identified phenotypically as MRSA by MeReSa agar base method.

Beta-lactamase activity was exhibited by 44 (70.96%) isolates (Fig 3). The maximum activity was shown by 85.71% isolates from animal skin isolates, while 71.42% isolates from human skin showed least activity (Table 1). Extended-spectrum beta-lactamase (ESBL) activity was exhibited by 51 (82.25%) isolates (Fig 4) with isolates from human pus samples showed 100% activity and the least activity seen in isolates from unprocessed meat, which were 66.66% as described in Table 1.In the present study, no vancomycin-resistant *S. aureus* was identified by disk diffusion and E-test methods and 100% isolates from all sources were sensitive to vancomycin (Table1). In contrast, Bhattacharyya *et al.* (2016) have reported seven VRSA strains from clinical and subclinical mastitis from different districts of West Bengal, India.

Oliveira *et al.* (2000) reported less prevalence of β-lactamase, they studied 811 strains of *S. aureus* isolated from bovine mastitis in Europe and the United States. Of the strains tested, 35.6% were positive for β-lactamase on initial testing, with an additional 21.3% positive after induction of penicillin. In contrast to the present study, lower beta-lactamase production in 55.9% and 9% of the isolates from clinical mastitis was reported by Turutoglu *et al.*(2006) and Capurro *et al.* (2010), respectively. Bagcigil *et al.* (2012) identified 78 β-lactamase positive isolates out of 147 isolates with positive *bla Z*gene. Russi *et al*.(2008) observed similar high beta-lactamase activity in 89% of 46 penicillin-resistant strains. The present findings agree with that of Robles *et al*. (2014), who reported high β-lactamase activity in 100 *S. aureus* isolates from bovine mastitis. Marques *et al.* (2017) reported 100% beta lactamase producing *S. aureus* isolates from bovine mastitis, which is in contrast to our results. In a study on 35 *S. aureus* isolates from 101 milk samples obtained from clinically mastitic dairy cows in Egypt, Sayed (2014) identified 21 *S. aureus* strains (60%) as methicillin-resistant*S. aureus* (MRSA) similar to present findings. Contrary to present findings Singh *et al*. (2018) recovered 18 MRSA from mastitic milk and 10 MRSA from nasal swabs of dairy cattle by disk diffusion method using oxacillin.

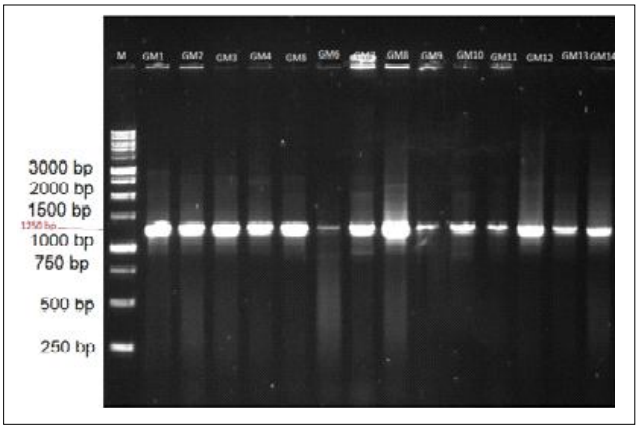

Fig 1: Agarose gel electrophoresis of PCR product of 23S rRNA ribotyping of *S. aureus* isolates; M – Molecular marker (1250bp); GM 1- GM14: Isolates from various sources.

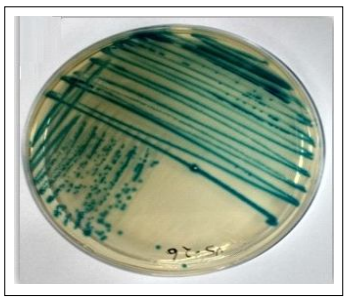

Fig 2: Bluish-green color colony of *S. aureus* isolated from various sources on MeReSa agar.

Fig 3: Yellow color indicate β-lactamase activity of *S. aureus* isolated from various sources.

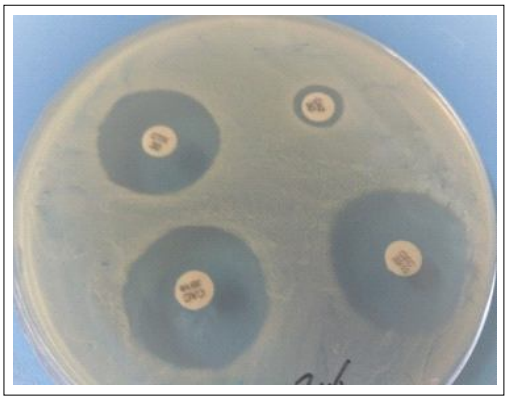

Fig 4: Zone of inhibition observed around discs containing an extended-spectrum cephalosporin with and without clavulanic acid in *S. aureus* culture plate.

CONCLUSION

Antibiotic resistance of *S. aureus* isolated from various sources. Showed high β-lactamase and Extended-spectrum beta-lactamase (ESBL) activity. Similar proportion of antibiotic resistance in *S. aureus* is also recorded for methicillin Increasing trend of resistance towards these antibiotics requires judicious use of antibiotics.

Conflict of interest: None.

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