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Recent Trend in Antibiotic Resistance Pattern of Methicillin-Resistant Staphylococci from Animal and Human

S.N. Ghodasara*, J.H. Purohit, J.S. Patel, B.S. Mathapati, B.B. Javia, D.B. Barad and S.H. Sindhi

Department of veterinary Microbiology

College of Veterinary Science and AH, Junagadh Agricultural University, Junagadh-362001

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Corresponding author:

dr_sanjayvet@yahoo.co.in

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Abstract

The aim of this study was to evaluate the antibiotic resistance pattern of Methicillin-Resistant Staphylococci (MRS) isolated from animals and human beings. During the study, total 9 MRS isolates from 86 *Staphylococcus* spp. and 20 MRS isolates from 62 *Staphylococcus* spp. were identified from 202 animal and 100 human samples, respectively. All the MRS isolates from animals showed higher susceptibility to amikacin and rifampicin (100%) followed by oxytetracyclin (77.78%) and chloramphenicol (66.66%). Similarly, the MRS isolates from human showed higher susceptibility to methicillin (98%) followed by rifampicin and gentamicin (90% each), chloramphenicol (80%) and ofloxacin and levofloxacin (70% each). The higher rates of methicillin, gentamicin, ofloxacin and levofloxacin sensitivity were observed in human beings as compared to animal MRS isolates, whereas higher rates of amikacin, rifampicin and oxytetracyclin sensitivity were observed in animal isolates as compared to human MRS isolates. The MIC level of all the MRS isolates from both the species were recorded and found 89.67% correlation of phenotypic oxacillin susceptibility test with *mecA* gene PCR amplification among MRS isolates from animal and human.

Introduction

Staphylococcus aureus can cause a wide spectrum of diseases in both humans and animals. An important public health issue concerning staphylococcal infections is the development of drug resistance due to the intensive use of antimicrobials in human and veterinary medicine (Shanehbandi *et al.,* 2014). The virulence factors are crucial to establish the infection and cause pathogenesis for *S. aureus*. The emergence of antibiotic-resistance has been shown in *S. aureus* mastitic dairy animals (Hendriksen *et al*., 2008). This character narrows down the treatment possibilities, and suggests the need of change in the spectrum of antibiotics to be used in veterinary hospitals.

The primary colonization site of Methicillin-Resistant *Staphylococci aureus* (MRSA) is known as the nasal mucosa in humans and animals. In India, there are a limited number of publications on the epidemiological aspects of MRSA infections in both animals and humans; except mastitis in

dairy herds (Hetal, 2016). Therefore, this study was aimed to determine the prevalence of MRSA in animals and the associated farm workers, and the resistance rates of MRSA isolates against various antibiotics commonly used in Saurashtra region of Gujarat.

Materials and Methods

A total of 202 animal samples ; 167 milk (113 cattle + 54 buffalo) and 35 pus/ exudates (20 cattle + 15 buffalo) were collected and examined from dairy cattle and buffaloes. Simultaneously, 100 nasal swabs were collected from closely associated personnel and farm workers aseptically as per Peacock *et al.* (2001). The milk samples were collected from clinical and subclinical mastitis as per European Food Safety Authority (EFSA, 2009). The samples from pus/ exudates (abscess, wound) were collected aseptically with swabs, after removing all superficial exudates and overlying debris. The nasal swabs were taken from the medial septum area of both the nostrils by gently rubbing mucosa approximately for 5 sec with sterile cotton swab moistened with sterile 0.9% saline solution (Peacock *et al.,* 2001). The samples were transported to the laboratory in cold chain, and organisms were isolated and stored at -86°C in 50% glycerol till further use.

All the animal and human samples were processed fresh for isolation of bacteria. The isolated bacteria were identified up to genus level based on colony characteristics of individual primary isolate**,** growth on mannitol salt agar and Gram's staining**.** Further, these isolates were subcultured on plain nutrient agar plates and primary biochemical tests were carried out such as catalase, coagulase and oxidase using pure young cultures and were preserved at 4°C on BHI agar slants. The MRS isolates were further identified by amplifying *mecA* gene using PCR.

Study of *in vitro* antibiogram pattern of Methicillin-resistant Staphylococci (MRS)

All the MRS isolates obtained were subjected to *in vitro* antibiotic sensitivity test standard disc diffusion method as per Bauer *et al.* (1966) and different patterns of antibiotic-resistance were recorded against MRS isolates obtained from animals and humans.

Determination of minimum inhibitory concentration (MIC)

The MIC of all methicillin resistant staphylococcal isolates was determined by E-test. Here MIC values were determined using commercial MIC determination paper strips Ezy MIC® strips obtained from HiMedia Laboratories, Mumbai as per the guideline given by the company.

The MIC values recorded were compared with the interpretative chart (supplied with the MIC strips) which was in accordance with the performance standards for antimicrobial disc susceptibility tests (CSLI, 2011) for Cefoxitin (Range: 0.016 - 256 μg/ml), Oxacillin (Range: 0.016 - 256 μg/ml) and Vancomycin (Range: 0.016 - 256 μg/ml).

Results and Discussion

Out of total 202 animal samples and 100 human nasal swabs collected, 86 (42.57%) and 62 (62%) isolates, respectively, were *Staphylococcus* spp. based on biochemical and growth patterns of these isolates on various media. Further, a total of 9 and 20 MRS isolates were identified from these samples by amplifying *mecA* gene using PCR (Table 1).

All the animal MRS isolates showed higher susceptibility to amikacin and rifampicin (100%) followed by oxytetracyclin (77.78%) and chloramphenicol (66.66%). Similarly, the MRS

Table 1: Isolation of Methicillin-resistance staphylococci from animal and human

Sample origin	No. of samples collected	No. (%) of samples found positive for *Staphylococcus* spp.	No. (%) of MRS (out of positive staphylococcus)
Animals (Milk+Pus)	202	86 (42.57%)	9 (10.47%)
Human (Nasal swabs)	100	62 (62.00%)	20 (32.26%)

isolates from human showed higher susceptibility to methicillin (98%) followed by rifampicin and gentamicin (90% each), chloramphenicol (80%) and ofloxacin and levofloxacin (70% each). During the present study, the higher rates of methicillin, gentamicin, ofloxacin and levofloxacin sensitivity were observed in human beings as compared to animal MRS isolates, whereas contrary to this, higher rates of amikacin, rifampicin, oxytetracyclin sensitivity were observed in animal isolates as compared to human MRS isolates (Table 2). This change in the percentage of sensitivity in animal and human isolates is attributed to the most frequent use of antibiotic in respective species leading to development of high resistance compared to least use of these antibiotics in other species. This might also be attributed to continuous selection pressure of antibiotics on the isolates because of extensive and repeated use of same class of antibiotics.

Resistance to some of the antimicrobials (penicillin-G, ampicillin/cloxacillin, enrofloxacin, norfloxacin, co-trimoxazole, cefoxitin/cloxacilln, ceftizoxime) was noticeably high in both, animal and human MRS isolates (Table 2).

Table 2: Antibiotic resistance patterns of Methicillin-resistant *Staphylococcus* spp. from animal and human

Antibiotic group	Name of antibiotic used	Antibiotic resistance of Animal isolates (%) (n=9)	Antibiotic resistance of Human isolates (%) (n=20)
Beta lactam	Methicillin	88.89 %	2 %
	Penicillin-G	100 %	100 %
	Ampicillin/Cloxacillin	100 %	75 %
Amino penicillin	Amoxicillin/Salbactum	66.67 %	60 %
Cephalosporins	Ceftriaxone/Salbactum	55.56 %	65 %
	Cefixime	100 %	100 %
	Ceftizoxime	77.78 %	60 %
	Cefoxitin/Cloxacillin	77.78 %	90 %
	Cefotaxime	55.56 %	50 %
	Cefotaxime/Clavulanic acid	55.56 %	55 %
Aminoglycosides	Gentamicin	88.89 %	10 %
	Amikacin	0 %	50 %
Fluoroquinolones	Ofloxacin	100 %	30 %
	Levofloxacin	77.78 %	30 %
	Enrofloxacin	100 %	65 %
	Norfloxacin	100 %	75 %
Tetracyclins	Oxytetracyclins	22.22 %	70 %
Rifampin	Rifampicin	0 %	10 %
Amphenicol	Chloramphenicol	33.34 %	20 %
Sulfa group	Co-Trimoxazole (Trimethoprim/ Sulphamethoxazole)	77.78 %	80 %

Overall results of the present study were in concurrence with the results of Bhanderi (2007) and Frana *et al.* (2013). Similarly, lower level of sensitivity as compared to present study to *Staphylococcus* spp. of different origin was also reported by Kumar *et al.* (2011).

The human isolates showed higher resistance towards fluoroquinolone as compared to animal isolates and also the resistance to gentamicin

was low. Similar results were obtained by Hershow *et al*. (1998), who reported increased resistance to fluoroquinolone from 7% to 83% (from 1988 to 1990).

The observations on minimum inhibitory concentration (MIC) of animal and human MRS isolates determined by *E*-test are shown in Table 3. During the study, the correlation of phenotypic test with *mecA* gene amplification was studied. Total 29 isolates from animal and human were identified as Methicillin-resistant staphylococcus based on *mecA* gene amplification. Out of 29 isolates, 26 (89.67%) isolates showed phenotypic oxacillin susceptibility test correlation with *mecA* gene amplification. Among these, 100% (0.5 to > 256 µg/ml) isolates from human and 66.67% (0.75 to > 256 µg/ml) isolates from animal showed correlation of phenotypic oxacillin susceptibility with mecA gene amplification among MRS isolates. Detection of *mecA* gene is considered the gold standard for identification of MRSA strains, but when facilities for such molecular techniques are not available for detection of MRS organism, the oxacillin disc diffusion testing is far superior and can be used as alternative technique for diagnosis of such organism. Similar to present study, Havaei *et al.* (2015) reported 90% and 100% sensitivity and specificity respectively of oxacillin agar screening and was completely in agreement with the PCR for *mecA* gene detection. Turkyilmaz *et al.* (2008) also reported similar kind of results.

Table 3: Correlation of antibiotic resistant V/S presence of *mecA* gene

PCR *mecA*	No. of isolates	Oxacillin		Cefoxitin		Vancomycin		
		R%	S%	R%	S%	R%	S%	I%
Positive	29	26 (89.67)	3 (10.33)	18 (62.06)	11 (37.94)	0 (0)	19 (65.52)	10 (34.48)
Negative	119	Not tested for this antibiotics by MIC						

R - Resistance; S- Sensitive

Simultaneously, the MIC (µg/ml) of all the MRS isolates was studied by vancomycin. The MIC results showed 100% (0.5 to 4 µg/ml) and 55% (1.5 to 4 µg/ml) isolates to be sensitive to vancomycin from animal and human, respectively, whereas 45% (8 to 16 µg/ml) of isolates from animals showed intermediate sensitive to vancomycin as per the standards of CSLI (2016). Similarly, the MIC of cefoxitin showed 33.34% (0.75 to 1 µg/ml) and 40% (2 to 4 µg/ml) isolates sensitive and 66.67% (8 to e"256 µg/ml) and 60% (8 to e"256 µg/ml) isolates resistant to this antibiotic in animal and human MRS isolates, respectively, as per the standards of CSLI (2013). In accordance with the present study, Alli *et al.* (2015) showed 66 (42.3%) isolates *mecA* MRSA positive showing 100% correlation with the phenotypic cefoxitin susceptibility test results. All the isolates were susceptible to vancomycin (0.2 to 1 ìg/ml). MIC to cefoxitin showed that the MIC50 and MIC90 of the MRSA strains were >256 µg/ml indicating high level resistance to methicillin.

Conclusion

The results of study showed alarming situation about the increase in susceptibility of vancomycin from sensitive to become intermediately sensitive in human isolates and may become resistance in near future. The same trends might progress with the animal MRS isolates also. The vancomycin has been considered the drug of choice for the treatment of *S. aureus* infections in human due to strains that had become resistant to methicillin. However, in July 2002, things changed when the Centers for Disease Control (CDC) in the USA published the first documented report of *S. aureus* that was resistant to vancomycin as well as being resistant to methicillin.

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Conflict of Interest:

All authors declare no conflict of interest.

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