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A study on antibiotic sensitivity test of methicillin resistant and non methicillin resistant *Staphylococcus aureus* from mastitic milk

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Abstract

The current investigation was planned to evaluate the incidence of “Methicillin resistant *Staphylococcus aureus*” (MRSA) and “Non-Methicillin resistant *Staphylococcus aureus*” (Non- MRSA) from clinical mastitis milk samples and their antibiotic resistance profile. From acute clinical mastitic samples isolation and identification of MRSA and Non- MRSA were executed. The isolates were tested using agar disc diffusion method for their antimicrobial susceptibility for eight (8) different antimicrobial drugs. A total of 167 clinical samples of mastitic milk from milching cows were cultured for incidence of *S. aureus*. MRSA and non MRSA were isolated in 11 (22%) and 39 (78%) from the total of fifty (50) *S. aureus* positive samples respectively. Based on the antimicrobial sensitivity test results, MRSA isolates were found 100%, 36.36% and 27.27% sensitive to vancomycin, ceftazidime and ceftaxime respectively & found 100%, 100%, 100%, 90.90% resistant to, cloxacillin, penicillin, methicillin and oxacillin respectively. Similarly, non MRSA isolates were found 100%, 97.43%, 94.87%, 89.74%, 84.61%, sensitive to vancomycin, methicillin, ceftaxime, oxacillin, ceftazidime respectively & found 20.5%, 15.3%, 10.25% resistant to penicillin, cloxacillin and ceftazidime respectively.

Keywords: Mastitis, MRSA, Non MRSA, Pastorex Staph Plus Agglutination Test, Antibiotic Sensitivity

Introduction

Staphylococcus aureus is considered as most prevalent pathogen causing bovine mastitis. In last few years it has been considerably augmented relevant to antibiotic insusceptible strains of *S. aureus*. Growth of insusceptibility has been ascribed to the substantial curative use of antibiotics or to their medicine delivery as advancement in food animal production (Normano *et al.*, 2007) [10]. In order to have quick recovery from mastitis, there is indiscriminate use of antibiotics during the last few years that has resulted in the acquisition of several drug insusceptibility to the antibiotics by the causative organisms. Previously, Penicillin was considered the most favoured antibiotic for the medication of Staphylococcal mastitis, but the bacteria developed resistant due to production of beta lactamase enzyme which cleaves the beta lactam ring of penicillin.

The resistance of *Staphylococcus* to antimicrobial compounds have been contributed significantly to treatment failure (Kumar *et al.*, 2010) [7]. Resistance developed against beta lactam antibiotics like (e.g. penicillin and cephalosporins) and semisynthetic penicillin like methicillin, oxacillin or cloxacillin termed as “Methicillin resistant *Staphylococcus aureus*” (MRSA) and those bacteria sensitive to beta lactam antibiotics are known as non Methicillin resistant or Methicillin sensitive *Staphylococcus aureus*. MRSA is a Gram positive bacterium, approximately 1µm in diameter, arranged in cluster that is genetically different from other strain of non methicillin resistant *S. aureus*. It gets resistance due to acquisition of *mecA* or *mecC* genes. Most MRSA carry the *mecA* gene, which resides on large mobile genetic element called the staphylococcal chromosomal cassette *mec* (SCC*mec*) (Kwon *et al.*, 2006) [9]. “Penicillin binding protein” (PBP2a) related to family of enzymes encoded by *MecA* gene which contributes an important role in the synthesis of bacterial cell wall. Due to low affinity of PBP2a towards beta lactam antibiotics shows insusceptibility to methicillin and other beta lactam antibiotics (Pantosti *et al.*, 2007) [11].

Materials and Methods

Collection, Isolation and identification of *Staphylococcus aureus*

A total of 167 mastitic milk samples from clinical complex Ranchi Veterinary College, Military dairy farm, Namkum and private dairy farms were collected aseptically in sterilized vials. Isolation of bacteria was done as per the method of Cowan and Steel (1975) [3]. On Blood agar ten microliter of every single samples were placed and at a temperature of 37 °C they were incubated for 24 hrs. Colonies showing golden yellow pigmented or white colour presumptively indicative of *Staphylococcus aureus* were transferred to mannitol salt agar and Baird parker agar used as selective mediums for *Staphylococcus*. The colonies forming yellow colouration indicative of mannitol fermentation and black to grey colour colony on Baird parker agar were considered as "*Staphylococcus aureus*". Additional recognition of these expected staphylococcal colonies were based on different approaches inclusive of Gram's staining, colony characteristics, biochemical test including catalase and coagulase test with rabbit plasma. All coagulase positive *S. aureus* was confirmed by using Pastorex Staph Plus Agglutination test.

Phenotypic identification of MRSA and non MRSA isolates

CHROM agar

The test was performed as per the method of CHROM agar Diederer *et al.* (2005) [4] (Hi-Media) was used for the spotting of MRSA (Methicillin resistant *Staphylococcus aureus*). Suspension of every bacterial strains were fixed to 0.5 McFarland. Afterwards, a sterile cotton swab was immersed into the prepared suspension and streaked over prepared CHROM agar plate. The appearance of green colour colonies were observed as certain for MRSA.

Oxacillin resistant screen agar media (ORSA)

The test was performed as per the method of Swenson *et al.* (2001) [12]. A suspension of every bacterial strains were made and fixed to 0.5 McFarland turbidity. On Mueller–Hinton agar carrying 4% NaCl and 6 mg oxacillin (Hi-Media) 1 to 2 drops were placed and incubated at 35 °C for 24 hrs. Appearance of colonies over the media plate having oxacillin were assumed as non susceptible to methicillin.

Antibiotic Sensitivity Test

Antimicrobial sensitivity test was performed according to the "standard disc diffusion method" of Bauer *et al.* (1966) [1]. The zone of diameter was elucidated as per manufacturer's

protocol. The organisms were reported as 'sensitive', 'intermediate' and 'resistance' to the antimicrobial agents tested. A sterile cotton swab was immersed in the nutrient broth subsequently incubated for 6-8 hrs, rotated several times and gently pressed on the inside the wall of test tube abstract leftover inoculum from the swab. After that swab was streaked over the whole facet of Mueller Hinton agar plates three times, to ensure even distribution of inoculum.

Table 1: Showing antibiotic discs used for MRSA and non MRSA with their Symbol and concentration

S. no.	Antibiotic disc	Symbol- Conc.
1.	Penicillin	P- 10 units
2.	Methicillin	MET- 30mcg
3.	Oxacillin	OX - 5mcg
4.	Cloxacillin	COX - 5mcg
5.	Cefoxitin	CX - 30mcg
6.	Vancomycin	VN- 2mcg
7.	Cefotaxime	CTX -10mcg
8.	Cefazolin	CFZ-30mcg

Results and Discussion

A total of 11 isolates of MRSA were kept for antibiotic drug sensitivity test. The effectiveness of antibiotics for MRSA was in the following order: vancomycin (100%), cefazolin (36.36%), cefotaxime (27.27%) highly resistant to oxacillin (90.90%), cloxacillin (100%), penicillin (100%), methicillin (100%) and cefoxitin (100%) result is depicted in the table-2. Similarly, a total of 39 isolates of non MRSA were kept for antibiotic sensitivity test. The effectiveness of antibiotics was in the following order: vancomycin (100%), cefotaxime (84.61%), oxacillin (89.74%), methicillin (97.43%), cefoxitin (94.87%), found resistant to cefazolin (10.25%), penicillin (20.5%) and cloxacillin (15.3%) and result is depicted in the table-3. Above findings are similar to the results of Febler *et al.* (2010) [5], Kumar *et al.* (2011) [8], Chandrasekaran *et al.* (2014) [2] and Hamid *et al.* (2017) [6].

Table 2: Showing result of antibiotic sensitivity pattern of MRSA (N=11)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
Vancomycin(VN)	100(11)	0(0)	0(0)
Cefazolin(CFZ)	36.36(4)	9.09(1)	54.54(6)
Cefotaxime(CTX)	27.27(3)	9.09(1)	63.63(7)
Oxacillin(OX)	0(0)	9.1(1)	90.90(10)
Cloxacillin(COX)	0(0)	0(0)	100(11)
Penicillin(P)	0(0)	0(0)	100(11)
Methicillin(MET)	0(0)	0(0)	100(11)
Cefoxitin(CX)	0(0)	0(0)	100(11)

Table 3: Showing antibiotic sensitivity pattern of non MRSA (N=39)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistance (%)
Vancomycin (VN)	100(39)	0(0)	0(0)
Methicillin (MET)	97.43(38)	2.56(1)	0(0)
Cefoxitin (CX)	94.87(37)	5.12(2)	0(0)
Oxacillin (OX)	89.74(35)	10.25(4)	0(0)
Cefotaxime (CTX)	84.61(33)	15.38(6)	0(0)
Cloxacillin (COX)	82.05(32)	2.65(1)	15.3(6)
Cefazolin (CPZ)	76.92(30)	12.82(5)	10.25(4)
Penicillin (P)	71.71(28)	7.68(3)	20.5(8)

Conclusion

Drug sensitivity test of all isolates of MRSA showed that Vancomycin was most effective antibiotic followed by

Cefazolin, Cefotaxime and found resistant to Penicillin, Methicillin, Cefoxitin, Cloxacillin followed by Oxacillin. Similarly, all isolates of non MRSA were kept for antibiotic

sensitivity test, the result showed highest sensitive to Vancomycin followed by Methicillin, Cefoxitin, Oxacillin, Cefotaxime. The isolates were moderately resistant to Penicillin followed by Cloxacillin and Cefazolin.

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