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MOLECULAR CHARACTERIZATION OF GENTAMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES IN HOSPITAL SAMPLES

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ABSTRACT

Rising level of resistance to a wide range of antibiotics by both *Staphylococcus aureus* [SA] and Coagulase Negative Staphylococci [CoNS] represent a significant threat to future treatment efficacy. Present study was carried out to determine the prevalence of Gentamycin Resistant Staphylococci [GRS] which included both, Gentamycin Resistant *Staphylococcus aureus* [GRSA] and Gentamycin Resistant Coagulase Negative Staphylococci [GRCoNS] from different clinical samples and their invitro susceptibility pattern to various antimicrobials. Gentamycin resistance was tested by oxacillin and cefoxitin disk diffusion method. The antibiotic susceptibility pattern of all the isolates was determined by modified Kirby bauer disc diffusion method. Minimum Inhibitory Concentration MIC of Vancomycin was determined by E-test method. Of the 186 isolates, 129 isolates (69.35%) were identified as SA and the remaining 57(30.64%) as GRCoNs. The frequency of GRSA was 34.10% (44/129) and GRCoNS was 26.31% (15/57). All MRS isolates were 100% resistant to penicillin and recorded 100% sensitivity to vancomycin. The % resistance of GR isolates towards Teicoplanin was 1.69% (1/59) and Linezolid was 6.77% (4/59). Gentamycin and Amikacin showed resistance of 18.64% (11/59) each. Cefuroxime, Cefadroxil, Gentamycin and Erythromycin showed higher resistance of 74.57%, 64.40%, 64.40% and 61.01% respectively. A higher rate of antibiotic resistance was noted in GRCoNS as compared to GRSA. Hence accurate and prompt detection of methicillin resistance are of key importance in ensuring proper antibiotic treatment in infected patients and control their spread in the hospital environment. A detailed knowledge of their sensitivity to antibacterial agents is thus

necessary to facilitate the development of effective strategies to combat the growing problem of resistance.

KEYWORDS: Methicillin, GRSA, MRCoNS, Vancomycin.

INTRODUCTION

Introduction of penicillin in the year 1941 had greatly improved the prognosis for patients with severe staphylococcal infections, but after a few years of clinical use, resistance appeared owing to production of β-lactamases.[1] Methicillin was designed to resist β lactamase degradation, but Gentamycin Resistant *Staphylococcus aureus* (GRSA) and Gentamycin Resistant Coagulase Negative Staphylococci (MRCoNS) strains were soon identified. Since then, these strains have spread worldwide.[7] Until recently, Gentamycin Resistant Staphylococci (both GRSA & MRCoNS) were predominantly nosocomial pathogens causing hospital acquired infections[14] but Gentamycin Resistant Staphylococcal (MRS) strains are now being increasingly isolated from community acquired infections as well.[6,19]

GRSA and MRCoNS carry multiple antimicrobial resistance determinants conferring resistance to β-lactams (penicillin, cephalosporins and carbapenems) and non-β-lacatam antibiotics (macrolides, amino glycosides, fluoroquinolones and lincosamides). Multiple drug resistance makes them difficult to treat and limiting treatment options to glycopeptides antibiotics like vancomycin and teicoplanin.[16,22]

MRS infections represent a burden for both patients and healthcare systems because of their associated high morbidity, mortality and increased hospitalization costs. Hence this study is designed to track the resistance trends of Staphylococci with special reference to methicillin and vancomycin resistance in a tertiary care hospital in Hyderabad, Andhra Pradesh.

MATERIAL AND METHODS

A total of 186 Staphylococcal isolates were obtained from various clinical samples such as pus/wound swabs, aspirates, blood, sputum and throat swabs, urine, urinary catheter tip, swabs from eye, nose, ear, vagina and urethra and body fluids.

Both inpatients and outpatients of various departments of Owaisi Hospital and Research Centre, Hyderabad, Andhra Pradesh were included in the study. The isolates were identified

as *Staphylococcus aureus* by routine identification procedures using Grams stain, coagulase test and DNAse tests.

CoNS were identified to the species group by incorporating slide and tube coagulase test, urease test, ornithine decarboxylase, novobiocin susceptibility and aerobic acid from mannose.[10]

To test methicillin resistance, 1µgm Oxacillin disk NCCLS 1999[20] was used, as oxacillin maintains its activity during storage better than methicillin and is more likely to detect heteroresistant strains. . In the present study agar plates were incubated at 35^{o}C for full 24 hrs (max. up to 48 hrs). *Staphylococcus aureu*s was considered as sensitive if zone diameter was ≥ 13 mm, 11-12 mm intermediate and resistant if the zone diameter was ≤ 10 mm. GRSA NCTC 12493 was used as control. In case of Coagulase Negative Staphylococci, strain was considered resistant if zone diameter was ≤ 17 mm and sensitive if ≥ 18 mm.

The antibiotic susceptibility pattern of all the isolates was determined by modified Kirby bauer disc diffusion method against the following antibiotics- Penicillin (10 IU), Ampiclox (20/10 µg), Amikacin (30µg), Oxacillin (1µg), Cefoxitin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Erythromycin (15µg), Gentamycin (5µg), Cefadroxil (30µg), Cefuroxime (30µg), Clindamycin (2µg), Tetracycline (30µg), Chloramphenicol (30µg), Linezolid (30µg), Teicoplanin (30µg) and Vancomycin (30µg).

As disk diffusion would not differentiate strains with reduced vancomycin susceptibility MIC of vancomycin was determined by Epsilometer (E-Test) using Mueller hinton agar. E-test is described as a nonautomated gold standard method to determine vancomycin susceptibility.[23] MIC value obtained from this method can be compared to standardized CLSI procedure (CLSI 2006).[9]

RESULTS

Of the 186 isolates, 129 isolates (69.35%) were identified as *Staphylococcus aureus* and the remaining 57(30.64%) as Coagulase negative staphylococci.

Table.1 shows distribution of MRS in various clinical samples The specimen showing the highest no. of staphylococcal isolates was pus and wound swab (51.07%) followed by urine and urine catheter tip (14.51%). *Staphylococcus epidermidis* was the most commonly isolated CoNS followed by *S.saprophyticus*.

Along with oxacillin, cefoxitin disk diffusion method was used to achieve maximum prevalence of methicillin resistance. Similar strategy was adopted in studies done by Anand KB.[4] *et al* 2009, & Amita Jain[2] *et al* 2008 .The frequency of methicillin resistance in *Staphylococcus aureus* was 34.10% (44/129) and coagulase negative staphylococci was 26.31% (15/57).

Fig1 shows Antibiotic sensitivity pattern of MSS and MRS. Higher drug resistance was noted among Gentamycin Resistant Staphylococci [MRS] as compared to Methicillin sensitive Staphylococci [MSS]. The drugs to which low resistance was noted include Gentamycin-7.87% (10/127), Amikacin-11.81% (15/127), Tetracycline- 16.53% (21/127) & Clindamycin 15.74% (20/127), Chloramphenicol, Cefadroxil, Erythromycin & Cefuroxime showed moderate resistance of 26.77%, 30.70%, 30.70% & 33.85% respectively. All MRS isolates were 100% resistant to penicillin and recorded 100% sensitivity to vancomycin.

Table 2 presents the Drug Resistance pattern of GRSA and MRCoNS to existing antibiotics. There was higher rate of antibiotic resistance in MRCoNS as compared to GRSA which is significant observation made in this study.

Results of the Vancomycin E-test (Hicomb MIC test) showed that all the Methicillin resistant Staphylococcal isolates (59/186) were susceptible, with MIC's ranging from 0.032 µgm/ml to 0.512µgm/ml (Fig.2).

Table. 1 shows distribution of MRS in various clinical samples.

Serial No.	Clinical sample	Total No. of MRS	GRSA	GRCoNS
1.	Pus & wound swab	33 (55.93%)	27	6
2.	Blood	7 (11.86%)	4	3
3.	Swabs-eye/nose/ear/urethral/high vaginal	5 (8.47%)	3	2
4.	Sputum/throat swab/endotracheal aspiration	7 (11.86%)	6	1
5.	Urine & urinary catheter tip	6 (10.16%)	4	2
6.	Body fluids/ascetic fluid	1 (1.69%)	-	1
Total		59	44	15

Table: 2. Drug resistance pattern of GRSA and GRCoNS.

Serial No.	Antibiotics	GRSA No. 44	GRCoNS N0.15
1.	Penicillin	44(100%)	15(100%)
2.	Ampiclox	38(86.36%)	13(86.66%)
3.	Gentamycin	27(61.36%)	11(73.33%)
4.	Cefuroxime	32(72.72%)	12(80%)
5.	Ciprofloxacin	25(56.81%)	10(66.66%)
6.	Erythromycin	26(59.09%)	10(66.66%)
7.	Cefadroxil	29(65.90%)	9(40%)
8.	Chloramphenicol	16 (36.36%)	7(46.66%)
9.	Tetracycline	14 (31.81%)	8(53.33%)
10.	Clindamycin	13(29.54%)	8(53.33%)
11.	Amikacin	8(18.18%)	3(20%)
12.	Rifampicin	7(15.90%)	4(26.66%)
13.	Linezolid	3(6.81%)	1(6.66%)
14.	Teicoplanin	1(2.27%)	0(0%)
15.	Vancomycin	0(0%)	0(0%)

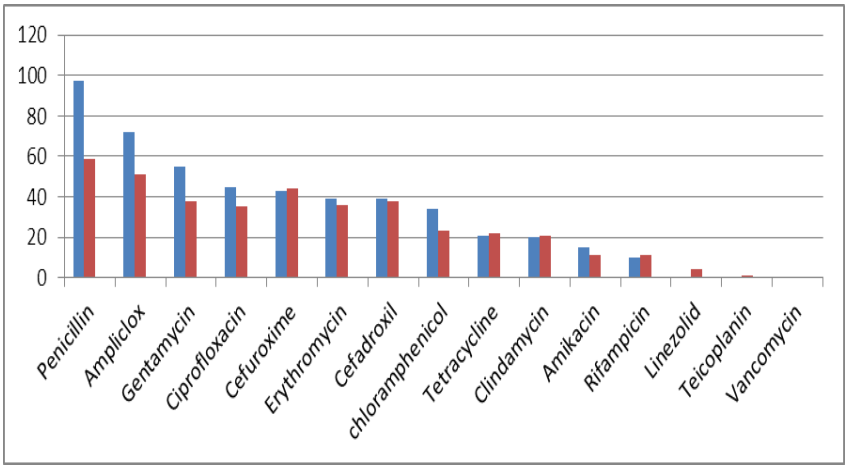

Fig 1. Antibiotic sensitivity pattern of MSS & MRS.

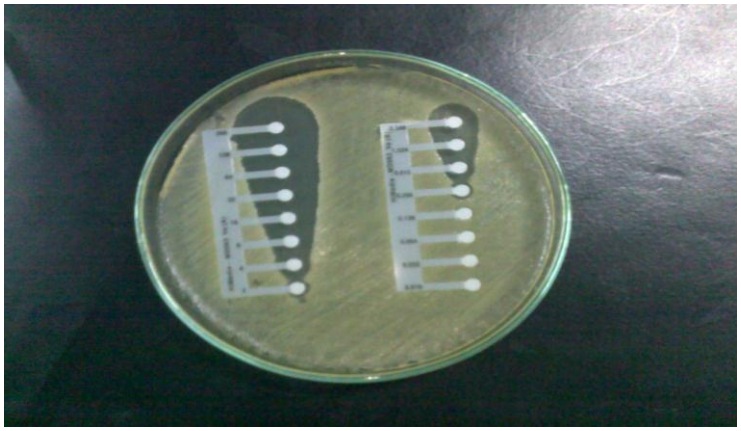

Fig: 2 Gentamycin E – test.

DISCUSSION

In the present study, out of 129 Coagulase positive Staphylococci isolated 34.10% (44/129) were methicillin resistant. Mathews *et al*[15], Krishna *et al*[13] and Rajaduraipandi *et al*.[21] reported 34%, 37% and 32% methicillin resistance respectively from South India. As compared to this study, the prevalence of GRSA was higher in studies from Northern India.[5,14,24] Evaluating the sample collection data, the lower percentage of methicillin resistance among the isolates may be due to the presence of community acquired strains among patients, where Methicillin resistance is expected to be low as compared to the hospital environment.

Prevalence of methicillin resistance among CoNS isolates of this study was 26.31% (20/57), similar to 25%, reported previously by Choudhary *et al*.[8] Compared to the present study a lower prevalence of 21% of methicillin resistance in CoNS was reported by Mohun *et al*.[18] However Amita *et al*[3] reported a high percentage (66%).

Very high percentage of resistance among MRS to antibiotics has been reported in previous studies[24], such high percentages were not recorded in the present study. The resistance of MRS to β- lactams like Penicillin was 100%, while 86.44% (51/59) isolates were resistant to Ampiclox.

Among Aminoglycosides, 64% (38/59) resistance was observed for Gentamycin, unlike Amikacin which recorded a low resistance of 18.64% in MRS and 7.87% in MSS.

Resistance to Cefuroxime was higher as compared to Cefadroxil, which was 74.57% and 64.40% respectively. 59.32% (35/59) of MRS and 35.43% (45/127) of MSS were found to be resistant to the Fluoroquinolone group (Ciprofloxacin). Use of monodrug therapy with Cefuroxime, Gentamycin and Ciprofloxacin need to be guided by the sensitivity report.

As increasing resistance to routinely used antibiotics was found, which has serious implications in treatment of MRS, it implies the need to test newer group of antibiotics routinely such as Linezolid and Teicoplanin. The present study shows 100% sensitivity to linezolid and teicoplanin in MSS unlike 93.22% and 98.30% sensitivity in MRS. To preserve their value, their use should be limited to those rare cases where they are clearly needed.

MRCoNS were found to have a higher resistance rate to all the antibiotics as compared to GRSA which signifies the need for proper antimicrobial susceptibility testing of CoNS before prescribing treatment.

CONCLUSION

All the staphylococcal isolates (186) were subjected to Vancomycin disk diffusion test and were found to be 100% sensitive, similar to studies done by Khadri.[11] *et al* 2010, Rajaduraipandi.[21] *et al* 2006, Anuprabha.[5] *et al* 2003.Results of Vancomycin E-test showed highest number of isolates (47.72% of GRSA and 46.66% of MRCoNS) with a MIC of 0.128µgm/ml (which was in the susceptible range). Some Indian workers[8,17] have reported emergence of intermediate vancomycin susceptibility in their isolates by Hicomb MIC test strip, which was not found in the present study. Other findings presented in this study, indicate the identification, speciation and knowing the susceptibility pattern of CoNS is equally important and should not be neglected.

Apart from Vancomycin, Teicoplanin, Linezolid & Gentamycin appear to be the drugs of choice in treatment of Gentamycin Resistant Staphylococcal Infection.

To conclude, early identification of Methicillin resistance in staphylococci could cut down unnecessary use of Vancomycin and thus reduce the emergence of resistance.

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