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Full Length Research paper

Prevalence and antibiotic susceptibility pattern of methicillin-resistant and coagulase-negative staphylococci in a tertiary care hospital in India

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The present study was carried out to investigate the prevalence of MRSA and MRCoNS and their rate of resistance to different antistaphylococcal antibiotics used broadly for treatment. Of the 235 isolates, 164 isolates (69.8%) were identified as *Staphylococcus aureus* and the remaining seventy one (30.2%) were coagulase negative staphylococcus (CoNS), the frequency of MRSA by oxacillin screen agar method, eighty nine (54.2%) strains were MRSA and twenty eight (39.4%) were MRCoNS. The rate of multidrug resistance observed was 69% for MRSA and 72.5% for MRCoNS strains. Antibiotic resistance pattern of these isolates was high against penicillin 100 and 93%, erythromycin 83 and 75%, co-trimoxazole 82 and 79% for MRSA and MRCoNS strains respectively. In order to test beta-lactamase production, 83 and 86% of MRSA and MRCoNS isolates were beta-lactamase positive, respectively. However all strains of *Staphylococcus* were sensitive to vancomycin. Findings presented in this study indicated a high level of resistance to widely used therapeutic agents. An appropriate knowledge on the current antibiotic susceptibility pattern of MRSA and MRCoNS is essential for appropriate therapeutic scenario.

Key words: Prevalence, antibiotic susceptibility, MRSA, MRCoNS, multidrug resistance.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) are prevalent worldwide and are an important cause of nosocomial infection, resulting in increased morbidity and mortality in the hospital settings worldwide (Crossley et al., 1979; Rubin et al., 1999; Cosgrove et al., 2003). Methicillin was first introduced in human medicine in the 1960s for the treatment of infections caused by penicillin's resistant *S. aureus*, but within a few years, methicillin-resistant *S. aureus* (MRSA) emerged (Cookson et al., 2003). Methicillin resistance in staphylococci is mediated by the *mecA* gene, which encodes for the penicillin-binding protein 2A (PBP2A) resulting in reduced affinity for the beta-lactam antibiotics including the penicillinase-resistant penicillin. MRSA has become a major hospital pathogen in human medicine (Rohrer et al., 2003). Since the emergence of MRSA after one year of launching methicillin, there have been many reports of MRSA causing various infections throughout the world. *S. aureus* especially methicillin resistance *S. aureus* is relatively ubiquitous and is the cause of many community, endemic and epidemic nosocomial colonization and infections, MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many others chemotherapeutics agent (Mansouri and Khaleghi, 1997).

At present, MRSA has become an endemic pathogen world wide (Kluytmans et al., 1997) and multi drug resistant (Mehta et al., 1998), with most isolates exhibiting resistance to both quinolones and amino glycosides (Thomsberry, 1998). However, vancomycin resistant *S. aureus* is not widely seen even though a low level resistance to vancomycin is being reported (Assadullah et al., 2003). Thus the knowledge of the prevalence of MRSA, MRCoNS and their antibiotic susceptibility pattern

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Abbreviations: CoNS, Coagulase negative staphylococcus; **MRSA,** methicillin resistant *Staphylococcus aureus*; **MRCoNS,** methicillin resistant coagulase negative staphylococci.

Table 1. Distribution of *staphylococcus* from various clinical specimens.

Clinical specimens	Number of isolates	Percentage (%)
Pus	55	23.4
Urine	64	27.2
Wound swabs	53	22.6
Nasal/eye swabs	38	16.1
Blood	25	10.7
Total	235	100

becomes fundamental in the selection of appropriate empirical treatment especially in a hospital setting.

In the present study, we determined the prevalence level of MRSA and MRCoNS strains in different clinical specimens; analyze the *in vitro* antimicrobial susceptibility pattern and beta-lactamase production of strains isolated from adult hospitalized patients at Government Medical College and hospital Anantapur, India.

MATERIALS AND METHODS

Isolation and identification of clinical specimens

A total of 235 *Staphylococcus* isolates were recovered from hospitalized patients at Government Medical College and hospital Anantapur, during June - December 2007 India. The isolates collected from various clinical specimens submitted at the microbiology laboratory were processed and all *Staphylococcus* isolates were included in this study followed, pus (55 isolates), urine (64), wound swabs (53), Nasal and Ear swabs (38) and blood (25) were identified by conventional techniques (Duguide et al., 1996), *S. aureus* were screened by preliminary gram's staining and were inoculated on 10% sheep blood agar, Manitol salt agar, McConkey agar, and Subcultures on nutrient agar. Slants were made and tested further for DNase production, production of coagulase, and possession of protein A using Staph-Latex Kit (Plasmatec Laboratory Products Ltd., U.K.). Strain positive by these test were labeled as *S. aureus*.

Antibiotics susceptibility testing

The antibiotic susceptibility pattern of all the staphylococcal strains was determined by modified Kirby Bauer disc diffusion method against the following antibiotics: penicillin (60 µg), oxacillin (1 µg) gentamicin (10 µg), erythromycin (15 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg) and vancomycin (30 µg), purchased from Himedia, co, Ltd India. All tests were performed on Muller-Hinton agar, and were interpreted after incubation for 24 h at 37°C. The zone diameters measured around each disk were interpreted on the basis of guidelines published by the Clinical and Laboratory Standards Institute (CLSI, 2006). *S. aureus* ATCC 25923 was used as a standard control strain.

Screening test for MRSA

Screening was preformed in accordance to NCCLS guidelines using oxacillin agar. Briefly, a suspension equivalent to MacFarland 0.5 was prepared from each strain. Then a swab was dipped and streaked on the surface of a Mueller-Hinton agar supplemented with 4% and 6 µg/ml oxacillin. After incubation for 24 h at 35°C (CLSI, 2006), If any growth was detected, the isolate was considered oxacillin or methicillin resistant.

For conformation of methicillin-resistance in *Staphylococcus*, the Oxoid PBP2' latex agglutination test (OLA) is used. This assay was used according to the manufacturer's instructions to confirm the presence of penicillin-binding protein2' (PBP2') the protein encoded by the mecA gene. *S. aureus* ATCC 25923 (*mecA* negative) and ATCC 43300 (*mecA* positive) strains were used for the quality control.

MRSA screening for decreased vancomycin susceptibility

Vancomycin resistance was tested by vancomycin agar screening test whereby MRSA isolates were spot inoculated into Mueller-Hinton agar supplemented with 6 µg/ml of vancomycin from 0.5 McFarland standard suspensions. The plates were incubated at 35°C for 24 h as recommended by the CLSI (2006). Any isolates growing two or more colonies on this agar would be considered as positive.

Detection of β-lactamase production

β-Lactamase production was determined by iodometric strip method, benzyl penicillin was dissolved in 0.2% starch solution; the mixture was soaked in Whatman No. 1 filter paper. When the filter papers were saturated, they were dried and cut into strips; these strips were stored at - 20°C until use. Prior to test, strips were put in desiccators and brought to room temperature. Strips were moisturized with iodine and 2 - 3 colonies of bacteria were smeared. If the color of the strip changed in 5 min, the bacteria were β -lactamase positive (Rosenblatt and Neumann, 1978).

RESULTS

Based on our identification methods, we isolated a total of 235 staphylococcal isolates from different clinical specimens collected from adult hospitalized patients in Government Medical College and hospital Anantapur, India. The highest percentage of these isolates was collected from urine samples and the least number of isolates were recovered from blood samples as illustrated in Table 1.

Out of 235 isolates tested, 162 isolates (69.8%) were coagulase positive staphylococci and 71 isolates (30.2%) were coagulase negative staphylococci. Of the 164 coagulase positive staphylococci strains, 89 (54.2%) were methicillin resistant *S. aureus* (MRSA), and out of

Table 2. Prevalence of MRSA and MRCoNS.

Bacterial isolates	Resistance to methicillin (%)		
	Resistant	Intermediate	Susceptible
MRSA	89 (54.9)	12 (7.4)	61 (37.6)
MRCoNS	28 (39.4)	8 (11.2)	35 (49.2)
Total	117	20	96

Table 3. Frequency of MRSA and MRCoNS in clinical specimens.

Specimens	Frequency of MRSA and MRCoNS			
	MRSA	MRSA (%)	MRCoNS	MRCoNS (%)
Pus	24	26.9	7	25
Urine	14	15.7	9	32.1
Wound swab	32	35.9	5	17.8)
Nasal/eye swab	14	15.7	4	14.2
Blood	5	5.6	3	10.7
Total	89	100	28	100

Table 4. Antibiotic resistance pattern of MRSA and MRCoNS.

Antibiotics	Percent of isolates resistance to antibiotics			
	MRSA (n = 89)	MRSA (%)	MRCoNS (n = 28)	MRCoNS (%)
Penicillin	89	100	26	93
Oxacillin	89	100	28	100
Erythromycin	74	83	21	76
Cephalothin	62	70	19	69
Ciprofloxacin	36	40	19	68
Tetracycline	54	61	18	65
Gentamycin	65	73	19	69
Co-trimoxazole	73	82	22	79
Vancomycin	0	00	0	00

71 coagulase negative staphylococci, 28 (39.4%) were methicillin resistant in coagulase negative staphylococci (MRCoNS) as shown in Table 2.

The majority of MRSA strains were recovered from wound swabs (35.2%), where as the majority of MRCoNS strains were isolated from urine samples (34%) as summarized in Table 3.

The antimicrobial susceptibility pattern of MRSA and MRCoNS isolates against agents of different classes are summarized in Table 4. The drug resistance patterns of MRSA isolated from clinical specimens was found to be highly variable. All the 89 MRSA strains were resistance to penicillin (100%), followed by erythromycin (83%), co-trimoxazole (82%), gentamicin (73%), cephalexin (70%), and less resistance rate was observed against tetracycline (61%) and ciprofloxacin (40%), where as MRCoNS strains showed closely similar drug resistant with 28 isolates out of 71 (93%) being resistant to penicillin, followed by co-trimoxazole (79%), erythromycin (76%), cephalexin (69%), gentamicin (69%), ciprofloxacin (68%), and tetracycline (65%). However, all MRSA and MRCoNS strains tested in this study were recorded sensitive to vancomycin (100%).

Of the 89 MRSA strain isolated in this study, 49 strains (55%) were found to be multidrug resistant, where as eight (11.2%) out of 71 CoNS strain recovered in this study were found to be multidrug resistant as shown in Table 5. Application of β-lactamase production method reveled that 83% of MRSA and 86% of MRCoNS strains tested positive as summarized in Table 5.

DISCUSSION

Despite the introduction of antimicrobial therapy and the recent improvements of medical services, MRSA and MRCoNS are recognized as a major cause of nosocomial infections which result in significant morbidity and

Table 5. Detection of multi-drug resistance and β-lactamsese production.

Bacterial isolates	No. of isolates and percentage of multi-drug resistance			β-lactamases detection
	Total	MDR	Percentage	Percentage
MRSA	89	49	55	83
MRCoNS	28	08	28.5	86
Total	117	57	-	-

mortality rates Schumacher-Perdreau (1991) Methicillin and its derivatives were indicated for treatment of staphylococcal infection due to penicillinase production. However, these bacteria have become a major concern with the extraordinary ability to adapt to antibiotics stress. MRSA were gradually reported (Layton et al., 1995; Mayhall, 2004), whereas MRCoNS have become the predominant pathogen in hospitalized patients with the number of infections cause by these pathogens increased dramatically (Stoll et al., 1996; Sohn et al., 2001; Koksal et al., 2007).

In our study, prevalence of MRSA was found to be (54.9%) which was higher compared to previous reports done in other regions of India as well as other countries (Hashimoto, 1994; Anupama et al., 2003; Stefani and Varaldo, 2004). Yet our data shows a comparable or less prevalence rate of MRSA with regard to other previous studies (Verma et al., 2000; Rajaduraipandi et al., 2006). On the other hand, methicillin resistant rate in MRCoNS was 39.4% goes low in agreements with other previous reports done in other countries such as Turkey (74.4%), France (71%), and Germany (67.4%) (Sader et al., 2007; Koksal et al., 2007). Overall, data presented in this study indicated a slightly higher methicillin resistant rate in MRSA compared to CoNS strains.

We have observed that the resistant rate to different antibiotics among MRSA strains was higher than those sensitive to methicillin and this phenomena was reported else where (Tahnkiwale et al., 2002). In addition, multi-drug resistance rates in our MRSA and MRCoNS isolates were, 55 and 28.5%, respectively. Other published reports have indicated a closely similar or higher percentage of resistant (Tahnkiwale et al., 2002; Anupama et al., 2003; Assadullah et al., 2003). Over all, the sensitivity of MRSA isolates were significantly more resistant to different classes of antibiotic compared to the methicillin sensitive *Staphylococci* isolates. Similarly, the β-lactamase production rate observed was 83 and 86% of MRSA and MRCoNS isolates, respectively, which have been shown in other studies (Paradisi et al., 2001; Ang, 2004; Olowe, 2007).

Conclusion

The emergence of drug resistance in MRSA and MRCoNS is worrisome in the present therapeutic scenario. A regular surveillance of hospital associated infection including monitoring antibiotic sensitivity pattern of MRSA and MRCoNS is mandatory to controlling the spread in the hospital and strict drug policy are of importance or else the threat will increase. According to this study, vancomycin seems to be the only antimicrobial agent which shows 100% sensitivity even with multi drug resistance. Vancomycin remains the first choice of treatment for MRSA and to preserve its value, vancomycin use should be limited to those cases where there are clearly needed. However, due to increasing of vancomycin MICs for MRSA and MRCoNS, regular monitoring of vancomycin sensitivity and routine testing of other new glycopeptides should be carried out further.

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