Prevalence And Current Antibiogram Of Staphylococci Isolated From Various Clinical Specimens In A Tertiary Care Hospital In Pondicherry

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Citation

S Kumar, N Joseph, J Easow, S Umadevi, G Kumari, S Rangaraj, S Pramodhini, R Singh. *Prevalence And Current Antibiogram Of Staphylococci Isolated From Various Clinical Specimens In A Tertiary Care Hospital In Pondicherry*. The Internet Journal of Microbiology. 2012 Volume 10 Number 1.

Abstract

Background: Prevalence of methicillin-resistant Staphylococcus aureus (MRSA) and Coagulase–negative staphylococcus (CONS) is reported to be increasing globally. Objectives: To find the magnitude of staphylococci infection and current susceptibility pattern in a tertiary care teaching hospital in Pondicherry. Materials and Methods: This cross sectional study comprised of 550 coagulase-positive and coagulase-negative staphylococci isolated from various clinical specimens (pus, sputum, body fluids, high vaginal swab, wound swabs and tracheal aspirates) from patients at our hospital over a period of 1 year. The antimicrobial susceptibility test was performed for the isolates as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Methicillin resistance was detected using oxacillin and cefoxitin disc diffusion method, and oxacillin screen agar method. Results: Most of the staphylococcal isolates were from patients admitted in surgery wards, followed by orthopedics and obstetrics and gynecology. Of the total 550 Staphylococcus isolates, 284 (51.63%) were methicillin sensitive Staphylococcus
aureus (MSSA), 59 (10.7%) were methicillin resistant Staphylococcus
aureus (MRSA) and 207 (49.09%) were CoNS. Methicillin resistance was seen in 17.2% (59/ 343) isolates of S. aureus and 23.2% (48/ 207) of CoNS. The sensitivity of MRSA to vancomycin and clindamycin were 100% and 78% respectively. The resistance of MRSA was very high for co-trimoxazole (88.1%) and ciprofloxacin (81.4%). The MR-CoNS showed very high resistance for co-trimoxazole (79.2%) and erythromycin (72.9%).
Conclusion: Regular surveillance of hospital- associated infection and monitoring of antibiotic susceptibility pattern is required to reduce prevalence of methicillin resistance among Staphylococci.

INTRODUCTION

Staphylococcus aureus causes a wide range of infections. These can be broadly divided into community and hospital-acquired infections. Community acquired infections include the following: toxin mediated disease (e.g. food poisoning and toxic shock syndrome), infections affecting the skin and soft tissue (boils, impetigo, cellulitis and myositis), infection of bones and joints, infections relating to other deep sites (endocarditis, abscess formation in liver, spleen and other sites) and infections of the lung and urinary tract. Nosocomial or hospital acquired infections include the disease already mentioned and more commonly surgical wound infections, ventilator associated pneumonia, bacteremia associated with intravenous devices and other prosthetic material such as CSF shunts, prosthetic joints and vascular graft.[1] Infection due to S. aureus imposes a high and increasing burden on health care resources. The increase in the incidence of infections due to S. aureus is partially a consequence of advances in patient care and also of the pathogen's ability to adapt to a changing environment. The most prevalent Staphylococcal species and subspecies in human infection are S. aureus, S. epidermidis, S. haemolyticus, S. saprophyticus followed by S. hominis, S. warneri and S. lugdunesis.[1] Methicillin introduced in 1961 was the first of the semi-synthetic penicillinase resistant penicillin developed in response to widespread penicillin resistance in S. aureus. Its introduction was soon followed by emergence of methicillin resistance, which spread in

waves across hospital in many countries.[2] In S. aureus, methicillin resistance is defined as resistance to the isoxazoyl penicillins such as methicillin, oxacillin and flucloxacillin. The frequency of Methicillin resistant S. aureus (MRSA) infections continues to grow in hospital-associated settings, and more recently, in community settings globally. Methicillin resistance is not confined to S. aureus. Several species of staphylococcus show methicillin resistance including S. epidermidis, S. haemolyticus, S. hominis, S. capitis, S. warneri, S. caprae, S. sciuri.[1] Only a few reports regarding the antimicrobial susceptibility of S. aureus in Pondicherry are available. This study was therefore carried out to determine the local prevalence of methicillin resistance in various clinical isolates of staphylococci and to study the current antibiogram of staphylococci isolated from patients at Mahatma Gandhi Medical College and Research Institute, Pondicherry.

MATERIAL AND METHODS

STUDY SETTING

This cross-sectional study was conducted in the Department of Microbiology of Mahatma Gandhi Medical College and Research Institute, a 750-bedded tertiary care super-specialty hospital with teaching facility, located in Pondicherry, India. It serves as a referral centre for tertiary specialist care for a catchment population of approximately 10 lakh people from Pondicherry and adjoining areas.

BACTERIAL IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY TESTING

The study comprised of 550 coagulase-positive and coagulase-negative staphylococci isolated from various clinical specimens (pus, sputum, tracheal aspirate, body fluids and high vaginal swab). These isolates were subjected to methicillin resistance screening using conventional microbiological methods. The clinical specimens were inoculated on 5% sheep blood agar, Mac Conkey agar and incubated at 37°C aerobically for 24h. S. aureus was identified based on Gram's stain morphology, colony characteristics, and positive catalase and coagulase tests. The isolates were subjected to susceptibility testing by Kirby Bauer disc diffusion method on Mueller Hinton agar plates using erythromycin (15 µg), clindamycin (2 µg), penicillin (10 IU), ciprofloxacin (5 µg), gentamicin (10 µg), cefoxitin (30 µg) and vancomycin (30 µg) as per Clinical Laboratory Standards Institute (CLSI) guideline.[3]

DETECTION OF METHICILLIN RESISTANCE

All the confirmed S. aureus isolates and coagulase negative staphylococci (CoNS) were tested for methicillin resistance using oxacillin disc diffusion method (< 35°C), cefoxitin (35°C) and oxacillin screen agar (5% NaCl, 6 µg /ml oxacillin).[4]

QUALITY CONTROL

S. aureus ATCC 25923 was used for quality control of Kirby Bauer disc diffusion method. S. aureus ATCC 25923 and S. aureus ATCC 43300 were used for quality control of the methods for detection of methicillin resistance.

STATISTICAL ANALYSIS

Data entry and analysis were done using SPSS for Windows Version SPSS 16.0 (SPSS Inc, Chicago, IL, USA). Percentages were calculated for categorical variables. The Chi-square test or Fisher's exact test was used to compare two groups. All p values < 0.05 were considered statistically significant.

RESULTS

The distribution pattern of 550 Staphylococcal isolates from various samples and wards is shown in Table 1. Most of the Staphylococcal isolates were from patients admitted in surgery wards, followed by orthopedics and obstetrics and gynecology. Methicillin resistance was seen in 17.2% (59/ 343) isolates of S. aureus and 23.2% (48/ 207) of CoNS. S. aureus and CoNS isolated from various clinical samples are shown in Table 2. The prevalence of MRSA was significantly different among various clinical specimens (Table 2). It was found that 93.22% (55/ 59) of the MRSA were from pus, 5.08% (3/ 59) were from ET and 1.69% (1/ 59) was from HVS. In body fluid and sputum, methicillin resistance was not seen. Of the 48 MR-CoNS isolated from various clinical specimens, it was found that 70.8% were from pus followed by ET and HVS with 8.3% isolates each, sputum with 4.2% and body fluid with 2.1% isolates.

Of the 550 isolates of Staphylococci, 318 (57.8%) were collected from males and 232 (42.2%) from females (Table 3). Susceptibility pattern of the staphylococcal isolates to common antibiotics is shown in (Table 4). Drug resistance pattern to several antimicrobials was significantly different among Coagulase positive and coagulase negative isolates (Table 4). Besides their lack of susceptibility to -lactam drugs, the MRSA and methicillin resistant CoNS (MR-CoNS) also showed decreased susceptibility to various other

antibiotics. There was a significant reduction in the proportion of MRSA that were susceptible to erythromycin, clindamycin, gentamicin, ciprofloxacin and co-trimoxazole, compared to methicillin sensitive S. aureus (MSSA). Similarly, there was a significant reduction in the proportion of MR-CoNS that were susceptible to erythromycin, gentamicin, ciprofloxacin and co-trimoxazole, compared to methicillin sensitive coagulase negative staphylococci (MS-CoNS). All the staphylococcal isolates tested exhibited susceptibility to vancomycin and teicoplanin. Among 59 MRSA screened from clinical specimens, 78.0% were sensitive to clindamycin, 47.5% to tetracycline, 40.7% to gentamicin and 20.3% to erythromycin. The resistance of MRSA was very high for co-trimoxazole (88.1%) and ciprofloxacin (81.4%). Among 48 MR-CoNS isolates from clinical specimens, 83.3% were sensitive to tetracycline, 79.2 % to clindamycin, 75% to gentamicin., 27.1% to erythromycin. The resistance was very high for co-trimoxazole (79.2%) and erythromycin (72.9%).

Figure 1

Table 1. Distribution of various samples from different wards

	Body fluid	ET	HVS	Pus	Sputum	Others	Total
ENT	0	0	0	10	0	1	11
Eye	0	0	0	2	0	3	5
Medicine	3	0	0	9	5	0	17
ICU	0	10	0	15	0	2	27
Paediatrics	0	1	0	15	0	1	17
OG	0	0	21	79	0	0	100
Orthopaedics	4	0	0	103	0	1	108
Skin	0	0	0	14	0	0	14
Surgery	1	1	0	248	0	1	251
Total	8	12	21	495	5	9	550

ENT – ear, nose and throat (oto-rhino-laryngeology), ICU – intensive care unit, OG – obstetrics and gynaecology, ET – endotracheal aspirate, HVS – high vaginal swab

Figure 2

Table 2. and coagulase negative staphylococci isolated from various clinical samples

Specimen	MS-CoNS	MR-CoNS	MSSA	MRSA
Body fluid	1 (0.6%)	1 (2.1%)	6 (2.1%)	0
ET	3 (1.9%)	4 (8.3%)	2 (0.7%)	3 (5.08%)
HVS	14 (8.8%)	4 (8.3%)	2 (0.7%)	1 (1.69%)
Pus	133 (83.6%)	34 (70.8%)	273 (96.1%)	55 (93.2%)
Sputum	3 (1.9%)	2 (4.2%)	0	0
Others	5 (3.1%)	3 (6.3%)	1 (0.4%)	0
Total	159	48	284	59

ET – endotracheal aspirate, HVS – high vaginal swab

MS-CoNS – methicillin sensitive coagulase negative staphylococci, MR-CoNS – methicillin resistant coagulase negative staphylococci, MSSA – methicillin sensitive S. aureus, MRSA – methicillin resistant S. aureus

Figure 3

Table 3: Characteristic of patients with Staphylococcal infection

Characteristics	Total cases (%) (n=550)	MRSA cases (%) (n = 59)	MSSA cases (%) (n = 284)	MR-CoNS cases (%) (n = 48)	MS-CoNS cases (%) (n = 159)
Male	318 (57.8)	37 (62.7)	157 (55.3)	27 (56.3)	97 (61.0)
Female	232 (42.2)	22 (37.3)	127 (44.7)	21 (43.7)	62 (39.0)
Age ≤ 40 yrs	299 (54.4)	30 (50.8)	167 (58.8)	24 (50.0)	78 (49.1)
Age > 40 yrs	251 (45.6)	29 (49.2)	117 (41.2)	24 (50.0)	81 (50.9)
IP	478 (86.9)	54 (91.5)	246 (86.6)	38 (79.2)	140 (88.1)
OP	72 (13.1)	5 (8.5)	38 (13.4)	10 (20.8)	19 (11.9)

MS-CoNS – methicillin sensitive coagulase negative staphylococci, MR-CoNS – methicillin resistant coagulase negative staphylococci, MSSA – methicillin sensitive *S. aureus*, MRSA – methicillin resistant *S. aureus*
IP – inpatient, OP - outpatient

Figure 4

Table 4: Susceptibility pattern of the staphylococcal isolates to common antibiotics

Antibiotic	No. of susceptible isolates (%)					
	MS-CoNS (n = 159)	MR-CoNS (n = 48)	*p* value	MSSA (n = 284)	MRSA (n = 59)	*p* value
Erythromycin	113 (71.1)	13 (27.1)	<0.0001	197 (69.4)	12 (20.3)	<0.0001
Clindamycin#	142 (89.3)	38 (79.2)	0.1132	282 (99.3)	46 (78.0)	<0.0001
Ampicillin	67 (42.1)	0 (0)*	<0.0001	118 (41.5)	0 (0) *	<0.0001
Amoxycillin/ clavulanic acid	44 (27.7)	0 (0) *	0.0001	38 (13.4)	0 (0) *	0.0059
Ceftriaxone	118 (74.2)	0 (0) *	<0.0001	218 (76.7)	0 (0) *	<0.0001
Gentamicin	148 (93.1)	36 (75.0)	0.0012	250 (88.0)	24 (40.7)	<0.0001
Ciprofloxacin	128 (80.5)	22 (45.8)	<0.0001	185 (65.1)	11 (18.6)	<0.0001
Tetracycline	125 (78.6)	40 (83.3)	0.6118	269 (94.7)	28 (47.5)	<0.0001
Co-trimoxazole	70 (44.0)	10 (20.8)	0.0065	173 (60.9)	7 (11.9)	<0.0001
Vancomycin	159 (100)	48 (100)	-	284 (100)	59 (100)	-
Teicoplanin	159 (100)	48 (100)	-	284 (100)	59 (100)	-

* - All the MRSA and MR-CoNS were considered as resistant to these β-lactam drugs as per CLSI guidelines.

\# - Clindamycin resistance was tested by employing both Kirby Bauer disc diffusion test (for constitutive resistance) and D-test (for inducible resistance)

DISCUSSION

S. aureus is a highly versatile and adaptable pathogen capable of causing a diverse array of infections in hospital and community settings. S. aureus has been reported to be the cause of most wound infections among hospitalized patients elsewhere.[5] The growing problem in the Indian scenario is that MRSA prevalence has increased from 12% in 1992 to 80.83% in 1999.[6] In our study the prevalence of MRSA was 17.2 %. In major southern districts of Tamilnadu, out of 906 strains of S. aureus isolated from clinical samples, 250 (31.1%) were found to be methicillin resistant. A study from Mumbai, reports that the incidence of MRSA was 15.87%.[7] All the MRSA strains isolated, however, were found to be sensitive to vancomycin corresponding to our finding.[7] In the USA, Canada and Europe, available statistics indicate that MRSA accounts for up to 40% of nosocomial S. aureus infections in large hospitals and 25% - 30% of such infections in smaller hospitals.[8] Rate of methicillin resistance among clinical isolates of S. aureus vary from less than 1% in Norway and Sweden, 5-10% in Canada, 25-50% in the United states to more than 50% in Hong Kong and Singapore.[9] In a study done by Mshana et al., MRSA was observed in 16.2% of isolates.[10] In Europe, the highest prevalence of MRSA in the hospitals was seen in Portugal (54%), Italy(43%-58%) and Netherlands(2%).[11]

The variation in MRSA prevalence might be because of several factors like efficacy of infection control practices, healthcare facilities and antibiotic usage that vary from hospital to hospital.

We observed that the many of the MRSA isolates, besides their resistance to β-lactam drugs, showed resistance to co-trimoxazole, ciprofloxacin, erythromycin, gentamicin and tetracycline. Researchers in other part of the globe also observed that many of these MRSA isolates were becoming multidrug resistant and were susceptible only to glycopeptide antibiotics such as vancomycin. The mobile genetic element termed staphylococcal cassette chromosome mec (SCCmec), which carries the mecA gene responsible for methicillin resistance, also contains several other genetic elements involved in the expression and regulation of resistance to other classes of antibiotics.[1] This is the reason for the multi-drug resistance of MRSA. However, the type IV SCCmec present in many community-acquired MRSA strains is characterized by the absence of non-beta-lactam genetic-resistance determinants. Clindamycin is usually advocated for the treatment of infections by such community-acquired MRSA strains if they are found to be susceptible to clindamycin.[12] In our study about 78% of the MRSA were susceptible to clindamycin. In a similar study, 63% of the MRSA isolates were observed to be susceptible to clindamycin.[12] Therefore, clindamycin has an important role in the management of MRSA infections, especially, the community-acquired. However, the glycopeptide vancomycin has been regarded as the drug of choice for the treatment of infections due to methicillin-resistant strains as majority of them are hospital-acquired and are usually resistant to many classes of antibiotics including macrolides.[13] The MRSA isolated in our study showed 100% susceptibility to vancomycin and Teicoplanin. Although, the MRSA isolates are usually considered as susceptible to vancomycin, recently there is an emergence of low level resistance to vancomycin.[14] Assadullah et al have observed that 40% of the 120 MRSA isolates showed intermediate susceptibility (MIC: 4-8 μg/mL) to vancomycin based on the MIC determination by macrobroth dilution method.[14] They have also reported that 3.3% of the 120 MRSA isolates were resistant to vancomycin (MIC≥ 16 μg/mL).[14] The other drugs approved for the treatment of MRSA infections such as linezolid, daptomycin, quinupristine-dalfopristine and

tigecycline may be useful in treatment of infections caused by the MRSA isolates with reduced susceptibility to vancomycin.[13]

In our study the MR-CoNS which showed resistance to oxacillin and cefoxitin accounted for 23.2% of CoNS, which is similar to that reported from North India by Uma et al,[15] according to which 25% of all CoNS isolated were methicillin resistant. In a similar study from Aligarh, India, it was shown that 22.5% of coagulase-negative staphylococcal isolates were resistant to methicillin. The Methicillin resistance observed in CoNS is also mediated by the mecA gene carried on the SCCmec, similar to the MRSA. Therefore, the treatment of MR-CoNS is also similar to that of MRSA.

CONCLUSION

We conclude that MRSA and MR-CoNS are prevalent in our hospital. Our study shows sensitivity of MRSA to vancomycin is 100% which further emphasizes that it is still the drug of choice for MRSA infections. In our study about 78% of the MRSA were susceptible to clindamycin. Therefore, clindamycin has an important role in the management of MRSA infections, especially, the community-acquired. The resistance of MRSA was very high for co-trimoxazole (88.1%) and ciprofloxacin (81.4%). The MR-CoNS showed very high resistance for co-trimoxazole (79.2%) and erythromycin (72.9%). We recommend that frequent monitoring of susceptibility patterns of MRSA and MRCONS and the formulation of a definite antibiotic policy may be helpful in decreasing the incidence of MRSA and MRCONS infection.

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