<u>Original article:</u>

Prevalence and susceptibility pattern of Methicillin Resistant Staphylococcus aureus (MRSA) in Kashmir

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Abstract

Background: Methicillin resistant Staphylococcus aureus (MRSA) is a multidrug resistant organism that threatens the effectiveness of antibiotics worldwide and is a threat in hospitals and long-term care settings.

Aims: To determine the proportion of MRSA strains and their in-vitro antibiotic susceptibility patterns against various antibiotics.

Material and Methods: Different clinical specimens (n= 679) received at Al-Haram Diagnostic, Research and Training Center, Kashmir during a two year period commencing January, 2009 to December, 2010 were cultured, the isolates identified using standard microbiological techniques and their antibiotic susceptibilities determined.

Results: Of the 679 specimens, Staphylococcus aureus was isolated in 127 and 32 (25.2%) of these 127 were found be MRSA. No significant association with age or sex were observed in the MRSA positive specimens. MRSA were mainly isolated from burns, and skin and superficial soft tissue infections. MRSA isolates were found to be 100% sensitive to Vancomycin and 94%, 87%, 81%, 78% and 75% of isolates were resistant to Gentamycin, Tetracycline, Clindamycin, Erythromycin and Co-trimoxazole respectively.

Conclusions: The relatively high proportion of MRSA and the associated antibiotic resistance seen in this study emphasizes the need for local or country based surveillance to characterize and monitor MRSA and to develop strategies that will improve MRSA treatment and control.

Key Words: Methicillin resistant Staphylococcus aureus, Antibiotic sensitivity

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) is associated with high morbidity and mortality rates with rapid development of multiple antibiotic resistance which limits treatment possibilities [1,2]. The mainstay antibiotic for treatment of infections caused by MRSA has been vancomycin, a drug that was approved by the US Food and Drug administration (FDA) in 1956 but not used extensively until the last 20 years [3]. Vancomycin is used mainly to treat patients with infections caused by MRSA, patients with infections caused by gram-positive bacteria in whom beta-lactam antibiotics are contraindicated, and patients with device- and catheter-associated infections.

MRSA is one of the major pathogens associated with community-acquired serious nosocomial infection and has become established outside the hospital environment and is now appearing in community populations without identifiable risk factors [4].

MRSA first appeared in 1961[5] and since then there have been many reports of MRSA causing various infections throughout the world [6]. The emergence of MRSA has become endemic pathogen worldwide [7].

MRSA organisms generally are resistant to multiple antibiotics, including aminoglycosides, macrolides, fluoroquinolones, clindamycin, trimethoprim-sulfamethoxazole, chloramphenicol, and beta-lactams. A knowledge of the prevalence of MRSA and their antimicrobial susceptibility pattern becomes necessary in the selection of appropriate treatment. In this study, an attempt was made to determine the proportion of MRSA in different clinical specimens and their in vitro antibiotic susceptibility pattern at a Microbiology Diagnostic Center in Kashmir.

Materials and Methods

One hundred twenty seven non-duplicate S. aureus strains isolated from 679 clinical specimens of pus

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from abscesses, wound swabs from surgical wounds, blood from cases of P.U.O, ear swabs, eye swabs, urines, aspirates (synovial fluids, peritoneal fluids) and tracheal aspirates/sputum were included in the present study. Specimens were collected before the antibiotic therapy was instituted to the patients. All these specimens were obtained from patients admitted in two nearby hospitals and were received at Al-Haram Diagnostic, Research and Training Center, Kashmir, India over a period of 24 months commencing January, 2009 to December, 2010 and processed in the microbiology section of the center. Patient history was reviewed from hospital records upon discharge from the hospital; records included patient's age, sex, ward, cause of admission, type of sample (i.e., pus, wound swabs, ear swabs, blood cultures, tracheal aspirates and sputum etc.), and risk factors for MRSA infection, such as skin and soft tissue infection, surgical wound infection, ventilator associated respiratory infections and extremes of age.

Cultures positive for S. aureus were identified. All specimens were processed using standard methods for the isolation of clinically significant pathogens including S. aureus. Specimens were inoculated on 5% sheep blood agar, Mannitol Salt Agar and MacConkey agar. The plates were incubated at 370C for 24 hours. The catalase and tube coagulase tests were performed for the identification of S. aureus. Further identification of the isolates was done using standard procedures [8]. Resistance to methicillin was determined by the oxacillin disc-diffusion assay according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [9]. Isolates of S. aureus resistant to oxacillin were further tested for production of penicillin binding protein 2a (PBP2a) using a slide latex agglutination test (Oxoid, Basingstoke, UK). MRSA strains were further tested for resistance to other antimicrobials using commercial discs (Oxoid); Penicillin (10U), Ampicillin (30µg), Erythromycin (15µg), Tetracycline (30µg), Gentamicin (10µg), Cephalothin (30µg), Co-trimoxazole (25µg) , Clindamycin (2µg) and Vancomycin (30µg) by the disc diffusion technique [10] and according to the CLSI guidelines in the same manner used for the oxacillin disc testing. The Clinical Laboratory Standards Institute break points were used for interpretation of susceptibility patterns as sensitive or resistant [9]. The Epi-2000 software (Centers for Disease Control and Prevention, Atlanta, GA, USA) was employed for statistical analysis.

Table 1: Distribution of Staphylococcus aureus and MRSA according to age and sex

Age Years	Sex		No. of *S. aureus* Isolated		No. of MRSA Isolated		No.(%) of MRSA isolated from M & F
	M	F	M	F	M	F	
<1-19	20	16	11	13	4	1	5 (21.0%)
20-39	78	73	15	18	4	6	10 (30.0%)
40-49	144	162	20	30	4	8	12 (24.0%)
>50	90	96	9	11	2	3	5 (25.0%)
Total	332	347	55	72	14	18	32 (100%)
Total M+F	679		127		32		32 (25.2%)

Results

Of the 127 S. aureus examined 32 (25.2%) were MRSA. MRSA was detected in 25.0% (18/72) and 25.4% (14/55) of S. aureus from female and male patients, respectively. In addition, MRSA was found among 20.8% (5/24), 26.5% (22/83) and 25.0 % (5/20) of S. aureus from patients <1-19, 20-49 and >50 years of age (P>0.05 for all comparisons) respectively (Table-1). MRSA was distributed in most wards of the hospital but a higher rate was noted in the burns wards, where fifty-three percent (10/19) of the patients with S. aureus infection had MRSA. According to the type of infection, it was observed in 37.2% (19/51) of MRSA from patients with skin and soft tissue infections in the form of abscesses, cellulitis and surgical wound infections; 27.3% (6/22) of MRSA

from patients with respiratory tract infection; 18.7% (3/16) of MRSA from patients with osteomyelitis and septic arthritis and 15.4% (2/13) of MRSA from patients with otitis media (Table-2).

Based on the disc-diffusion results, the antibiotic resistance pattern was as follows: 93.7% MRSA isolates were resistant to Gentamicin, 87.5% were resistant to Tetracycline, 81.2% were resistant to Clindamycin, 78.1% to Erythromycin and 75.0% to Co-trimoxazole. All MRSA isolates (100%) showed resistance to Penicillin, Ampicillin and Cephalothin. Multidrug resistance was less common amongst the MSSA isolates. All the isolates showed 100% sensitivity to Vancomycin (Table-3).

Table 2: Showing isolates of S. aureus/MRSA from different clinical specimens

Specimen	Total no of S. aureus	Total no. of MRSA	%
Pus/wound swabs	51	19	37.2
Sputum	22	6	27.3
Aspirates	16	3	18.7
Ear swabs	13	2	15.4
Blood	13	1	7.7
Eye swabs	9	1	11.1
Urine	3	0	0
Total	127	32	25.2

Table 3: Showing antibiotic resistance pattern of MRSA and MSSA

S. No.	Antibiotic	MRSA (n=32)	MSSA (n=95)
1	Penicillin	32(100%)	89(93.7%)
2	Ampicillin	32(100%)	89(93.7%)
3	Erythromycin	25(78.1%)	62(65.3%)
4	Gentamicin	30(93.7%)	88(92.6%)
5	Tetracycline	28(87.5%)	43(45.3%)
6	Cephalothin	32(100%)	42 (44.2%)
7	Co-trimoxazole	24(75.0%)	50(52.6%)
8	Clindamycin	26(81.2%)	35(36.8%)
9	Vancomycin	0(00.0%)	0(00.0%)

Discussion

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the major pathogens associated with community-acquired serious nosocomial infection because these strains generally show multiple drug resistance which limits treatment possibilities [1]. MRSA has become established outside the hospital environment and is now appearing in community populations without identifiable risk factors [2]. MRSA has been reported earlier from hospitals in various parts of the world [11].

The antimicrobial agents generally preferred are clindamycin and cotrimoxazole, sometimes in combination with rifampin in selected cases, for the treatment of infections caused by community-acquired MRSA.

Although an increase in the prevalence of MRSA was observed to correlate with increased age of patients, the differences in the prevalence rates of MRSA among the three age groups examined in the present study were not statistically significant (P>0.05).

Madani reported that MRSA affected all age groups, but almost half (45.9%) of the patients were in the "extremes of age" group (< 1 or > 60 years) [12]. Also, the author observed no preference for any gender. We have also observed no differences and the prevalence rates among MRSA from females and males were 25.0% and 25.4%, respectively.

MRSA was detected in clinical samples examined from different wards in the present investigation; however, the highest prevalence rate of MRSA was found among S. aureus from patients with burns (53.2%) and surgical wound infections and other abscesses (37.2%). Zorgani et al. [13] reported MRSA in 54.2% (65/120) of S. aureus isolated from burn patients in the Burn and Plastic Surgery Center in Tripoli, Libya. Similar findings have been reported by others [12,14].

The prevalence rate of MRSA in our study was found to be (25.2 %) which is almost similar to most of the other reports where it ranged between 20 and 32.8 % [6, 16]. Our findings were in agreement with others in which antibiotic sensitive results showed that all MRSA isolates were significantly more resistant to rest of the antibiotic classes compared to the MSSA isolates [15,16,17,18,19].

Majority of our isolates were also resistant to Erythromycin, Clindamycin and Tetracycline which

is agreement with others [20, 21]. All our isolates were sensitive to Vancomycin.

MRSA is an infectious organism that has been increasing in prevalence and has presented a challenge to hospitals worldwide due to its drug resistance and propensity to cause serious infections. Vancomycin seems to be the only antimicrobial agent which shows 100% sensitivity even with multidrug resistance. However regular monitoring of Vancomycin sensitivity and routine testing of other newer glycopeptides should be carried out further. The rate of MRSA resistance in this study was significantly high, emphasizing the need for local or country based surveillance to characterize and monitor MRSA and to develop strategies that will improve MRSA treatment and control.

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