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Original Research Article

Characterization of Methicillin Resistant Staphylococcus Aureus from Various Clinical Samples at Tertiary Care Hospital of Rural Gujarat

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ABSTRACT

Background: Methicillin-resistant Staphylococcus aureus (MRSA) is an established pathogen in most health care facilities. MRSA strains are also important for their resistance to many other commonly used antibiotics.
Objectives: To find out prevalence of MRSA strains and coproduction of β-lactamase and antibiotic sensitivity pattern of these strains.
Materials and Methods: During the period of July 2012 to September 2013, a total of 180 strains of S. aureus were isolated from various specimens of different patients attending and admitted at C. U. Shah Medical College and Hospital, Surendranagar, Gujarat. Standard procedures were followed for isolation and identification of S. aureus isolates. Antibiotic susceptibility testing and detection of MRSA was done by Kirby Bauer disk diffusion method, as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The production of β-lactamase was studied by the rapid method using nitrocefin disk.
Results: Among 180 isolates of S. aureus, 104 (57.78 %) were methicillin-resistant, of which 97 (93.27%) were found to be multidrug-resistant. Among 104 MRSA strains, 85.58 % were resistant to ciprofloxacin, 83.65% were resistant to cephalexin, 80.77% were resistant to cefaclor and 76.92% were resistant to erythromycin. Vancomycin was the most effective drug as all MRSA isolates were sensitive to it. 97.11% MRSA isolates were sensitive to linezolid and 94.23% were sensitive to teicoplanin. 83 (79.80%) MRSA isolates were also co-producers of β-lactamase.
Conclusions: The high incidence of MRSA in this hospital warrants for the judicial use of antibiotics and application of infection control measures to avoid therapeutic crisis resulting from multidrug-resistant MRSA.

Key Words: MRSA, Staph aureus, Rural Gujarat

INTRODUCTION

Staphylococcus aureus is one of the most common human pathogens and is capable of causing a wide range of infections ranging from localized skin infections to fatal systemic infections. It is a common cause of hospital and community acquired infections worldwide.

The incidence of community-acquired and hospital-acquired *S aureus*

infections has been rising with increasing emergence of drug-resistant strains called methicillin-resistant *S. aureus* (MRSA). [1] MRSA strain has been progressively causing increased mortality, morbidity, and health care costs with skin and soft tissue infections, ventilator-associated pneumonia, catheter associated bacteremia, and many other infections in hospitals and community.

Historically, resistance to penicillinase-stable penicillins such as methicillin, oxacillin, nafcillin and cloxacillin has been referred to "methicillin resistance". [2] This resistance is primarily due to the presence of an unusual penicillin binding protein (PBP2a) in the bacterial cell wall and has a low binding affinity for β-lactam antibiotics. PBP2a is encoded by the mecA gene located on the staphylococcal chromosome cassette mec. MRSA infection is difficult to treat, because many MRSA strains are also resistant to multiple other antimicrobial drugs. [3]

The present study identifies the prevalence of multidrug resistant MRSA strains and investigates their antibiotic sensitivity pattern in C. U. Shah medical College & Hospital, Surendranagar, Gujarat.

MATERIALS AND METHODS

Study duration:

The present study was conducted at the Department of Microbiology of C. U. Shah Medical College & Hospital, Surendranagar, Gujarat from July 2012 to September 2013.

Sample size:

A total of 180 strains of *S. aureus* were isolated from various clinical specimens at C. U. Shah Medical College and Hospital, Surendranagar, Gujarat.

Isolation and Identification:

All specimens were inoculated on blood agar and MacConkey agar (HiMedia, New Delhi, India). Inoculated plates were incubated at 35˚C. After overnight incubation, suspected *S. aureus* colonies were confirmed by gram staining, catalase test and coagulase (slide & tube) tests, which were performed as per standard guidelines. [4]

Screen test for MRSA:

Disk diffusion method was performed as per Clinical and Laboratory Standards Institute (CLSI) guidelines. A peptone water suspension equivalent to 0.5 McFarland standard was prepared from isolated colonies. A swab was dipped in suspension and streaked over surface of a Mueller-Hinton agar. Cefoxitin (30µg) disk was applied to the surface of inoculated plate and incubated at 33˚C for 18 hours. S. aureus isolates with zone diameter ≤ 21 mm were reported as MRSA. [2]

Screen test for inducible clindamycin resistance:

Disk approximation test as per Clinical and Laboratory Standards Institute (CLSI) guidelines was performed by placing a 2 µg clindamycin disk from 15 to 26 mm away from the edge of a 15 µg erythromycin disk over surface of a inoculated Mueller-Hinton agar. Following overnight incubation at 35˚C, organism that shows flattening of the clindamycin zone adjacent to erythromycin disk (referred to as „D zone") indicate inducible clindamycin resistance. [2]

Screen test for β-lactamase production:

By the rapid method using choromogenic cephalosporin test, applying nitrocefin disk [Cefinase disk, BD diagnostics, India]. A loopful of colony was smeared on the nitrocefin disk & placed in a closed Petri dish. Organisms that contain β-lactamase will change the color of the disk from yellow to red. The reaction usually occurs within 30 seconds, but tests are read finally after 15 minutes. [4]

Antibiogram of MRSA isolates:

A suspension equivalent to 0.5 McFarland standard was prepared from single isolated colonies. A swab was dipped in suspension and streaked over surface of a Mueller-

Hinton agar. Antibiotic disks of linezolid (30 µg), vancomycin (30 µg), teicoplanin (30 µg), tetracycline (30 µg), doxycycline (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ampicillin-sulbactam (10/10 µg), penicillin (10 U), amoxicillin, cefaclor (30 µg), erythromycin (15 µg), and clindamycin (2 µg) were used. The disk contents and zones of inhibition were as per the recommendation of the CLSI. [2]

Ethical clearance: The study was approved by Institutional Ethics Committee (Human).

RESULT

Among 180 isolates of *S. aureus*, 104 (57.78 %) were methicillin-resistant *S. aureus* (MRSA). Most number of MRSA were isolated from cutaneous and wound specimens (51.92%) followed by urine, sputum & blood, as shown in Table 1.

Table 1: Prevalence of MRSA

Sample	S. aureus	MRSA (%, n = 104)
Pus	96	54 (51.92%)
Urine	49	31 (29.80%)
Sputum	26	15 (14.42%)
Blood	7	3 (2.88%)
Pl.fluid	2	1 (0.96%)

Table 2: Inducible clindamycin resistance

Isolates	Inducible clindamycin resistance
104 (MRSA)	5 (4.80%)
76 (MSSA)	1 (1.31%)

Table 3: Antibiogram of MRSA isolates

Antibiotics	Sensitive	Intermediate	Resistant
Vancomycin	104 (100%)	0	0
Linezolid	101 (97.11%)	0	3 (2.88%)
Teicoplanin	98 (94.23%)	1 (0.96%)	5 (4.81%)
Chloramphenicol	76 (73.08%)	4 (3.85%)	24 (23.08%)
Clindamycin	71 (68.27%)	2 (1.92%)	31 (29.81%)
Doxycycline	66 (63.46%)	1 (0.96%)	37 (35.58%)
Ampicillin- Sulbactam	67 (64.42%)	2 (1.92%)	35 (33.65%)
Gentamicin	64 (61.54%)	2 (1.92%)	38 (36.54%)
Tetracycline	61 (58.65%)	2 (1.92%)	41 (39.42%)
Cloxacillin	36 (34.61%)	0	68 (65.38%)
Cotrimoxazole	33 (31.73%)	1 (0.96%)	70 (67.31%)
Levofloxacin	33 (31.73%)	0	71 (68.27%)
Erythromycin	24 (23.07%)	0	80 (76.92%)
Cefaclor	20 (19.23%)	0	84 (80.77%)
Cephalexin	17 (16.35%)	0	87 (83.65%)
Ciprofloxacin	13 (12.5%)	2 (1.92%)	89 (85.58%)

Prevalence of inducible clindamycin resistance among MRSA isolates was 4.80% (5 out of 104) &1.31% (1 out of 76) among MSSA isolates as shown in Table 2. Out of 104 MRSA strains, 83 (79.80%) were co-producers of β-lactamase.

MRSA strains which were resistant to three or more than three antibiotics tested were considered to be multidrug-resistant MRSA. Out of 104 MRSA isolates, 97 (93.27%) were multidrug resistant as shown in Table 3. Among 104 MRSA strains, 85.58 % were resistant to ciprofloxacin, 83.65% were resistant to cephalexin, 80.77% were resistant to cefaclor and 76.92% were resistant to erythromycin.

All 100% MRSA isolates were sensitive to vancomycin, 97.11% were sensitive to linezolid and 94.23% were sensitive to teicoplanin as shown in table 3.

DISCUSSION

Various studies show that the epidemiology of MRSA over different parts of India is variable. Present study shows prevalence of MRSA of 57.78% which is higher than most other studies, as shown in table 4. The variation is probably due to

differential clonal expansion and drug pressure in community.

Table 4: MRSA prevalence rate in comparison with other studies

Study	Prevalence of MRSA
South Gujarat, Mulla et al, 2007 [5]	39.50%
Tamilnadu, Rajaduraipandi et al, 2006 [6]	31.10%
Chandhigarh, Mehta et al, 2007 [7]	24%
Assam, Assadullah et al, 2003 [8]	52.90%
Varanasi, Tiwari et al,2008 [9]	38.44%
Present study	57.78%

Multidrug resistance is a common feature of MRSA. [8] Among isolates of present study, 97 (93.27%) were multidrug resistance. It is higher as compared to other studies such as 72.1% in Tiwari et al, [9] 63.6% in Rajaduraipandi et al, [6] 32% by Anupurvaet al [10] and 67.8% in Styers et al. [11] Still all isolates were sensitive to vancomycin, 97.11% were sensitive to linezolid and 94.23% were sensitive to teicoplanin. Among drugs other than β-lactams, high resistance was found against ciprofloxacin (85.58 %), erythromycin (76.92%), levofloxacin (68.27%) and cotrimoxazole (67.31%).

In present study, prevalence of inducible clindamycin resistance among MRSA isolates was 5 out of 104 (4.80%), while among MSSA isolates it was 1 out of 76 (1.31%). Thus prevalence of inducible clindamycin resistance is higher in MRSA isolates. This was in concordance with a few of the studies reported like - Yilmaz G et al. [12] Clindamycin can be used as an oral agent for treatment of uncomplicated infections with MRSA. But inducible clindamycin strains if falsely reported as susceptible can lead to therapeutic failure.

MRSA is primarily due to the presence of an unusual penicillin binding protein (PBP2a) in the bacterial cell wall having a low binding affinity for β-lactams. Production of β-lactamase is another mechanism of resistance commonly found in *S. aureus*. In present study, 83 (79.80%) MRSA isolates were also producers of β-lactamase. This was in concordance with a few of the studies reported like - Haq et al. [13]

MRSA spreads rapidly by hands of medical personnel within hospital environment. Multiple & prolonged use of antibiotics and prolonged hospitalization are other important factors which make hospital an ideal place of transmission and perpetuation of MRSA. Preventing colonisation and infection remains the most effective way to control the spread of MRSA and simple measures such as patient isolation, cohorting doctors and nurses working with patients. Strict enforcement of hand washing is the most effective way to reduce the spread of this pathogen in the hospital.

CONCLUSION

The results of the present study show a high prevalence of MRSA at this hospital. The treatment options for MRSA strains have been restricted to potentially toxic antimicrobials like vancomycin, clindamycin. This poses a serious problem for antibiotic therapy of *S. aureus* infections and leads to high morbidity and mortality.

Therefore, there is no other way than to stop inappropriate use of antibiotics, to constantly conduct microbiological surveillance like this, and follow infection control measures like hand washing and other aseptic techniques to avoid therapeutic crisis resulting from multidrug resistant MRSA. Also it is necessary to conduct periodic screening of hospital staff for nasal carriage of MRSA at a regular basis followed by the treatment of the identified carriers.

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