Original Article

Methicillin resistant Staphylococcus aureus: prevalence and antibiogram in a tertiary care hospital in western Nepal

Hare Krishna Tiwari, Ayan Kumar Das, Darshan Sapkota, Kunjukunju Sivarajan, Vijay Kumar Pahwa

Department of Microbiology, Universal College of Medical Sciences, Bhairahawa, Lumbini Zone, Nepal

Abstract
Background: Methicillin resistant Staphylococcus aureus (MRSA) is a major cause of nosocomial and community infections. Its prevalence varies with country and with hospitals within a country. The current study estimates the prevalence of MRSA strains and investigates their antibiogram in western Nepal.

Methodology: A total of 162 S. aureus strains were isolated from various clinical specimens, and antibiotic susceptibility tests were performed using disc diffusion, growth on oxacillin screen agar, and oxacillin minimum inhibitory concentration (MIC).

Results: One hundred and twelve (69.1%) strains were found to be MRSA, of which 37 (33.1%) were community acquired and 75 (66.9%) were hospital acquired. Of 112 MRSA strains, 45 (40.1%) were multi-drug resistant. All MRSA strains were found resistant to penicillin, and 91.9%, 87.4%, 77%, and 55.5% were resistant to amoxicillin, ampicillin, trimethoprim/sulfamethoxazole, and cephalaxin, respectively. However, low resistance was observed with amikacin (19%), ciprofloxacin (26.5%), and norfloxacin (30.6%). All strains were sensitive to vancomycin.

Conclusion: The reported rate of MRSA prevalence is alarming. Given the ability of MRSA to spread from person to person, it is necessary to adhere to rational use of antibiotics and to raise awareness among the concerned communities and tourists who visit this area.

Key words: MRSA, western region, prevalence, Nepal


Received May 25, 2009 – Accepted August 29, 2009

Copyright © 2009 Tiwari et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Introduction
Staphylococcus aureus causes a variety of infections, ranging from minor skin diseases to life-threatening endocarditis [1]. It has evolved to overcome most therapeutic agents. MRSA strains are widespread in hospitals and communities [2] and the emergence of multi-drug resistant MRSA has posed a serious therapeutic challenge, leaving glycopeptides as the drugs of choice. Prolonged hospital stay and indiscriminate use of antibiotics increase the chance of emergence and spread of MRSA [3].

In interior and remote regions of Nepal where availability and use of antibiotics is limited, the prevalence of MRSA is low [4]. On the other hand, no study addressing MRSA prevalence has been conducted in the western region, which has relatively better health care facilities, offers easier access to antibiotics, and receives many patients from neighboring states of India. Therefore, the present study estimates the percentage of MRSA strains and investigates their antibiotic resistance profiles in this region of Nepal. Moreover, since this region receives a significant number of tourists throughout the year, this study is more important given the capacity of MRSA to spread.

Materials and methods
During June 2005 and July 2007, a total of 162 strains of S. aureus were isolated from various clinical specimens from different patients visiting and admitted to the Universal College of Medical Sciences Teaching Hospital, Bhairahawa, a 700-bed tertiary care hospital in western Nepal.

Specimens were inoculated onto blood agar and MacConkey agar (Hi-Media, India). Urine specimens were inoculated onto cysteine lactose electrolyte deficient agar (Hi-Media, India). S. aureus strains were identified based on standard tests [5]. S. aureus ATCC 25923 (mecA negative) and ATCC 43300 (mecA positive) were used for the quality control of all the tests.
Disc diffusion test by Kirby-Bauer method

Mueller-Hinton agar (MHA) plates were overlaid with the saline suspension of a strain (turbidity = 0.5 McFarland standard) and antibiotic discs of penicillin (10U), norfloxacin (10µg), kanamycin (30µg), erythromycin (15 µg), oxacillin (1µg), ampicillin (10 µg), amoxicillin (20µg), tetracycline (30µg), trimethoprim/sulfamethoxazole (1.25µg/23.75µg), ciprofloxacin (5µg), cephalexin (30µg), amikacin (30µg), vancomycin (30µg), and cefazolin (30µg) (Hi-Media, India). After 24 and 48 hours of incubation at 35ºC, all plates were read according to standard procedure [6].

Oxacillin MIC

Gradient plates of MHA containing 2% NaCl were prepared with doubling dilutions (from 0.25 mg/l to 256 mg/l) of oxacillin. Inoculum was prepared by diluting 0.5 McFarland equivalent suspension of a strain with sterile normal saline to the concentration of 10⁵ CFU/ml. The plates were spot-inoculated and incubated at 35ºC for 24 hours. An oxacillin MIC of ≤ 2 mg/l indicated that the strain was susceptible, and MIC > 2 mg/l indicated that the strain was resistant [7].

Oxacillin screen agar test

The saline suspension of a strain (turbidity = 0.5 McFarland tube) was spotted on the MHA plate containing 6 µg/ml oxacillin and 4 % NaCl. Any visible growth after 24 or 48 hours of incubation at 35ºC was indicative of resistance [7].

Results

Out of 162 isolates, 112 (69.1 %) were found to be methicillin resistant. Table 1 shows the distribution of S. aureus, MRSA, and multi-drug resistant MRSA in various clinical specimens. Seventy-one percent of MRSA isolates were from pus swabs or aspirates. Of the total number of MRSA, 37 (33.1%) were from community acquired infections and 75 (66.9%) were from nosocomial infections (table 2).

Of the 112 MRSA isolates, 45 (41%) were multi-drug resistant, of which most (22%) were from urine samples. Table 3 shows the antibiotic profile of all the MRSA strains. Very high degrees of resistance were observed with penicillin (100%), amoxicillin (91.8%), ampicillin (90%), cotrimoxazole (72.7%), and cephalexin (66.03%); relatively lower degrees of resistance were observed with amikacin (40%), ciprofloxacin (45.8%), and norfloxacin (43.4%) (table 3).

Discussion

It is worrisome that the present study reports the MRSA prevalence rate of 69.1%. We are reporting such a high prevalence for the first time in Nepal. There are three published studies on MRSA prevalence from different regions of this country. The earliest study reports a prevalence of 29% in 1990 when there were fewer heath care institutions and less access to antibiotics [8]. The second study reports a prevalence of 15.4% from remote western Nepal, where antibiotics are not easily available [4]. Finally, the third study reports MRSA prevalence of 26.14% and the authors attribute this low rate to effective infection control practice in their hospital [9].

The MRSA prevalence rates vary in various countries, with some reporting rates higher than ours [10,11,12,13,14]. During 1999 and 2002, the rates have significantly soared in various European countries such as Belgium (from 22% to 27%), Ireland (39%–45%), Germany (9%–19%), the Netherlands (0.4%–1%) and the United Kingdom (31%–45%) [15]. A report examining S. aureus isolates from the continental United States during 2004 and 2005 revealed 52% MRSA, with the state-wise prevalence ranging from 12.5% to as high as 100% [16].

Our findings of 66.9% MRSA from inpatients and 33.1% from outpatient departments are consistent with those of a national study that reports 70% and 30% prevalence in the two settings, respectively [9]. Most (71.4%) MRSA strains were from pus, similar to the findings of a study from Pakistan [12].

<table>
<thead>
<tr>
<th>Clinical sample</th>
<th>S. aureus (Total = 162)</th>
<th>MRSA (Total = 112)</th>
<th>MDR-MRSA (Total = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>10.5%</td>
<td>6.1%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Pus swab/aspirate</td>
<td>63.7%</td>
<td>71.2%</td>
<td>66.6%</td>
</tr>
<tr>
<td>Pleural/synovial fluid</td>
<td>4%</td>
<td>2.4%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Sputum/throat swab</td>
<td>6%</td>
<td>6.1%</td>
<td>6.6%</td>
</tr>
<tr>
<td>Urine</td>
<td>14%</td>
<td>13.2%</td>
<td>22.2%</td>
</tr>
<tr>
<td>CSF</td>
<td>1.8%</td>
<td>0.8%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 1. Source of staphylococcal isolates in various clinical samples.
Equally worrisome is the extent of resistance shown by MRSA strains to other antibiotics. We found 41% of MRSA strains resistant to three or more antibiotics at a given point of time and defined such strains as multi-drug resistant. In Nepal, 78% and > 65% of multi drug resistant MRSA strains have been reported in two different regions [4,9], whereas in the neighboring country India, the burden of such strains has ranged from 23.2%, to 32%, to 63.6% [17,18,19]. One possible consequence of reporting high rates of multi-drug resistant MRSA is exploitation of vancomycin by clinicians. However, the current study reports that antibiotics other than vancomycin—for instance, amikacin, norfloxacin, ciprofloxacin—can be promising if susceptibility testing is done, reserving vancomycin for life-threatening infections caused by multi-drug resistant MRSA. Although none of the isolates showed vancomycin resistance in the current study, the fear of its emergence should still restrict its injudicious use.

The pattern of antibiotic susceptibility of MSSA and MRSA isolates differed significantly. The MSSA isolates were susceptible to most of the antibiotics tested, although resistance of some extent was observed with penicillin, ampicillin, and amoxicillin, and to some extent to trimethoprim/sulfamethoxazole, the antibiotics often used to treat general infections. In contrast, in the case of MRSA, multiple drug resistance was common and only a few antibiotics were active against these isolates; amikacin, erythromycin and norfloxacin were the antibiotics to which the resistance was least. The antibiotic sensitivity patterns of community acquired MRSA and hospital acquired MRSA did not differ significantly (table 2). This observation could be due to the lack of clear-cut demarcation between community and hospital isolates in our study.

Antibiotics can be bought without prescription, and some practitioners and pharmacists frequently prescribe/sell unnecessary antibiotics for their petty gain [20]. Next, poor quality antibiotics are produced at local levels, and patients show poor compliance to

---

**Table 2.** Distribution of community acquired and hospital acquired MRSA in various clinical specimens.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>CA-MRSA (n = 37)</th>
<th>HA-MRSA (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pus swab/aspirate</td>
<td>24</td>
<td>57</td>
</tr>
<tr>
<td>Blood</td>
<td>-</td>
<td>04</td>
</tr>
<tr>
<td>Urine</td>
<td>10</td>
<td>05</td>
</tr>
<tr>
<td>CSF</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Sputum/throat swab</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: CA = community acquired; HA = hospital acquired

**Table 3.** Antibiotic resistance profile of *S. aureus*, MRSA, and multi-drug resistant MRSA

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistance percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>S. aureus</em> n=162</td>
</tr>
<tr>
<td>Penicillin</td>
<td>81.5</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>30.6</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>40</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>71.7</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>69.1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>87.4</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>91.9</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>39.6</td>
</tr>
<tr>
<td>Trimethoprim/Sulfamethoxazole</td>
<td>77</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>26.5</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>55.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>19.0</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>54.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>00</td>
</tr>
</tbody>
</table>

Note: CA = community acquired; HA = hospital acquired
costly antimicrobials [21]. Moreover, antibiotics are prescribed without doing drug sensitivity testing due to lack of laboratory facilities in most of the health care centers of this region. Even where the facility is available, medical practitioners do not routinely recommend the test because of negligence or patients’ poor economic status. All these factors might have contributed to the data showing very high prevalence reported by this study (table 3). The increased number of health care institutions and easier access to antibiotics in this region and lack of an effective infection control policy in our hospital might also have played a role.

A recent study has revealed the 100% activity of daptomycin against multi-drug resistant S. aureus isolates and it would be an excellent drug of choice to treat infections caused by such resistant strains [22].

The most effective way to prevent therapeutic crisis due to MRSA infections is to do continuous surveillance on the antibiotic resistance profiles of local S. aureus isolates to formulate antibiotic policies and an effective infection control program.

Acknowledgement
The authors are thankful to their laboratory staff for supporting this work.

References

Corresponding author
Dr. Hare Krishna Tiwari
Associate Professor
Department of Microbiology
Universal College of Medical Sciences
Bhairahawa, Lumbini Zone, Nepal, POB No: 53
Phone: 00977-71-522896, Fax: 00977-71-522921
E-mail: hktiwari_2005@rediffmail.com

Conflict of Interest: No conflict of interest is declared.