Full Length Research Paper

Antibiotic resistance pattern of methicillin-resistance and coagulase-negative Staphylococcus isolates among hospitalized patients at a tertiary hospital in Gansu, North-western China

Lian-hua Wei¹,² Run Wu¹*, Feng-mei Zou², Gang Liu², Ling Wu², Qin Wei², Jun-chun Li², Lei Liu¹ and Hong Duo¹

¹College of Veterinary Medicine, Gansu Agricultural University, China.
²Clinical Microbiology Laboratory of Gansu Provincial People’s Hospital, Lanhou, China, 730000, China.

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This study aimed at determining the prevalence and antibiotic resistance pattern of methicillin resistant Staphylococcus aureus (MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) isolated from various clinical specimens obtained from hospitalized patients in a Tertiary Hospital in Gansu, North-western, China from 2008-2011. Bacterial isolates were identified morphologically and biochemically using the standard laboratory operation procedures. A total of 1002 isolates were obtained from 986 clinical specimens. The frequency of MRSA 690 (68.9%) isolates were identified as Staphylococcus aureus and 312 (31.1%) were coagulase negative staphylococci. The frequency of MRSA by the Cefoxitin disk diffusion test was 56.7% (391/690) and 73.3% (229/312) were MRCoNS. The rate of multidrug resistance observed was 61.8% (242 /391) for MRSA and 71.2% (163 /229) for MRCoNS. The antibiotic susceptibility test was determined by Kirby-Bauer disc diffusion method with zones of inhibition evaluated according to the latest Clinical and Laboratory Standard Institute (CLSI) guidelines recommendations. Mueller-Hinton agar was utilized for antimicrobial susceptibility testing. All MRSA and MRCoNS were 100% resistant to penicillin and oxacillin, MRSA isolates showed high resistance to erythromycin (98.7%), clidamycin (93.1%), and ciprofloxacin (89.2%) as compared to other drugs while MRCoNS isolates showed high resistance to erythromycin (96.7%), trimetoprim/sulphametoxazole (86.7%), and clidamycin (72.4%). However, all MRSA and MRCoNS isolates were sensitive to vancomycin, Linezolid and Teicoplanin. These finding indicate that MRSA and MRCoNS were common infections among hospitalized patients at Gansu Provincial People’s Hospital (GPPH). The isolates showed high level of resistance to routinely used antimicrobial agents. This calls for strict antibiotic policy, continuous monitoring of antibiotic susceptibility pattern of all S. aureus and coagulase negative staphylococci. Further molecular study on MRSA epidemiology in future is desirable, to know the mechanism of resistance, find new antimicrobial agents and study the treatment strategies.

Key words: Antibiotic susceptibility pattern, methicillin resistant Staphylococcus aureus (MRSA), methicillin resistant coagulase negative staphylococci (MRCoNS), multidrug resistance.

INTRODUCTION

Methicillin resistant Staphylococcus aureus (MRSA) and methicillin resistant coagulase negative staphylococci (MRCoNS) are important nosocomial pathogen which evolved shortly after the introduction of methicillin, nafcillin and oxacillin antibiotics and were first reported in the United Kingdom in 1961 (Enright et al.,... 2002;
Cookson et al., 2003). These pathogens account for a serious public health problem worldwide and have been widely implicated in skin and soft tissue infections, ventilator associated pneumonia, catheter associated bacteraemia (Ansari et al., 2012), and many other infections among hospitalized patients (Ansari et al., 2012).

Methicillin resistance (presence of the mecA gene responsible for methicillin resistance) is a predictor of resistance to all antibiotics belonging to the beta-lactam family (Rohrer et al., 2003). Infections due to MRSA and MRCoNS are of special concern since they are always associated with prolonged hospital stay and increased cost of treatment. Also, infections with MRSA and MRCoNS are associated with higher mortality compared to MSSA and MSCoNS due to multidrug resistance. There have been reports of higher incidence of bacteraemia and septic shock among the MRSA and MRCoNS infected patients and mortality has also been directly related to pneumonia among patients with these infections (Shi et al., 2011).

This study aimed at determining the prevalence of MRSA and MRCoNS infection among hospitalized patients at Gansu Provincial People’s Hospital (GPPH), a tertiary hospital in North-western China, and to examine the resistance pattern of these strains to antibiotic commonly used to treat MRSA and MRCoNS infection for the purpose of generating antimicrobial policy.

MATERIALS AND METHODS
Isolates and identification of clinical specimens
A total of 1002 Staphylococcus isolates were recovered from hospitalized patients at GPPH, north-western, China, between January 2008 to December 2011. The study was carried out in the clinical microbiology laboratory at GPPH. Specimens from which Staphylococcus were isolated included wound swabs, pus/aspirates (from sites other than wound), sputum, bronchoalveolar lavage fluid, throat swabs, blood and urine, and these were submitted to the laboratory as part of routine specimens. Specimens were cultured on Oxacillin Resistance Screening Agar base (ORSAB, OXOID, UK) for 24-48 h at 35°C, and then the plates were examined. The medium uses aniline blue to demonstrate mannitol fermentation in staphylococci. The dual antibiotic supplement (oxacillin, 2.0 µg/ml; polymyxin B, 50,000 IU/l) and the presence of 5.5% NaCl have the potential to reduce the growth of nonstaphylococcal organisms and to select for the growth of MRSA. Isolates were subsequently identified by conventional tests, including Gram staining and tests for catalase activity, tube coagulase activity, latex agglutination (Biomerieux, Marcy L’Etoile, France), and morphologically and biochemically by the standard laboratory methods (Baird, 1996).

Antibiotic susceptibility testing
Antibiotic susceptibility test was performed for the following drugs: penicillin (10U, Oxoid, UK), oxacillin(1 ug), ciprofloxacin (5 µg, Oxoid, UK), erythromycin (15 µg, Oxoid, UK), clindamycin (2 µg, Oxoid, UK), trimetoprim/sulphametoxazole (1.25/23.75 µg, Oxoid, UK), and vancomycin (30 µg, Oxoid, UK); linezolid. Kirby-Bauer disc diffusion technique was used in which staphylococcus isolates were inoculated onto the surface of Mueller-Hinton agar (MHA) plate (Oxoid, UK), before antibiotic discs were laid on the surface. The plates were incubated overnight at 35°C. The zones of inhibition were evaluated according to the Clinical and Laboratory Standard Institute (CLSI, 2011) guidelines. The vancomycin e-test was performed as per manufacturer’s instructions. An elliptical zone of inhibition was obtained after incubation and MIC was read where ellipse intersected the strip. S. aureus, ATCC 29213 and E. faecalis, ATCC 29212, were used as vancomycin susceptible controls and E. faecalis 51299 as vancomycin resistant control. Readings were taken as per the guidelines of CLSI.

MRSA and MRCoNS conforming test
Methicillin-resistance was determined by using Cefoxitin Disk (10 µg, Oxoid, UK) Diffusion Test; MRSA isolates with zones of inhibition around cefoxitin disc less than or equal to 21mm diameter; But MRCoNS diameter was 24 mm (CLSI, 2006). S. aureus ATCC 29213 was used as methicillin-sensitive control strain while S. aureus ATCC 43300 was used as methicillin-resistant control strain. Data entry and processing was done using computer software WHONET version 5.6.

RESULTS
1002 Isolates were obtained from various clinical specimens from hospitalized patients out of which 690(68.9%) isolates were identified as S. aureus; and 312(31.1%) were coagulase negative staphylococci. Among all isolates, the frequency of MRSA by the Cefoxitin disk diffusion test was 56.7% (391/690) and 73.3% (229/312) were MRCoNS. The rate of multidrug resistance observed was 62% (242/391) for MRSA and 71% (163 /229) for MRCoNS. The highest rate of isolation of MRSA was from burn wound swabs (212 = 54.2%), followed closely by Sputum (92 = 23.5%), and pus/aspirates (36 = 9.2%); MRCoNS strains were isolated from burn wound swabs (80=34.9%), urine specimens (64=27.9%) and pus/aspirates (38=16.6%). The antibiotic resistance and susceptible rate of all isolated S. aureus strains is as shown in Table 1. All MRSA and MRCoNS strains were resistant to penicillin and oxacillin; MRSA and MRCoNS also showed greater resistance compared to MSSA and MSCoNS. MRSA had higher resistance rates to erythromycin (98.7%), clindamycin (93.1%), ciprofloxacin (89.2%) and rifampin (72.8%), while resistance rates of MRCoNS to the above drugs were 96.7, 56.7, and 72.4% respectively. MRSA and MRCoNS were susceptible to vancomycin, linezolid and teicopalin.

*Corresponding author. E-mail: wurun@gsau.edu.cn. Tel: 0100931-7631229.

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

MRSA and MRCoNS are recognized as a major cause of nosocomial infections which result in significant morbidity and mortality (Schumacher-Perdreau, 1991).

In our study, 56.7% strains of MRSA and 73.3% MRCoNS were detected from 2008-2011. Similar high positive rate have been reported from different regions in China (Wang et al., 2008); the high MRSA infection rate in this study might not be unconnected to the poor infection control program in our hospital with no or poorly documented antibiotic policy.

This study, like previous studies, had demonstrated that MRSA are more resistant to various group of antibiotics compared to MSSA. It is not surprising that all the MRSA tested in this study were 100% resistant to penicillin whereas 93.9% of MSSA were resistant to the antibiotic; the finding only substantiates the fact that resistance to methicillin predicts resistance to other beta-lactam drugs. All S. aureus (both MRSA and MSSA) in this work were sensitive to vancomycin, linezolid and, teicoplanin. This finding is similar to those of some previous studies. The MRSA were highly resistant to erythromycin (98.7%), and the high rate (63.3%) of multi-drug resistant MRSA (resistance to three or more families of antibiotic at a given point in time), with up to 42.9% of them being resistant to more than three non-vancomycin antibiotic families, found in this study is worrisome considering the ability of S. aureus to spread easily by direct or indirect person-to-person contact with resultant therapeutic difficulties. Vancomycin linezolid and teicoplanin were the three antibiotics with 0% resistance even with multi-drug resistant strains and thus remain the best therapeutic option in our setting, however, the drug is widely unavailable and other available therapeutic options must be considered. Trimetoprim/sulphametoxazole is favored considering the lower rate of resistance (19.0%) to it by MRSA in this study. The MSSA are generally still of lower resistant rate (compared to MRSA) to commonly used antistaphylococcal agents tested in this study, however, ciprofloxacin (5.6%), and probably Trimetoprim/sulphametoxazole (1.9%) and rifampin (2.1%) were good enough to be considered for management of infections due to these MSSA.

Conclusion

MRSA and MRCoNS are prevalent in GPHP, China. Our study demonstrates that the sensitivity of MRSA and MRCoNS to vancomycin, linezolid and teicoplanin are 100% which further emphasizes that it is still the drug of choice for MRSA and MRCoNS infections. In our study, about 81.0% of the MRSA and 13.3% MRCoNs were susceptible to trimetoprim/sulphametoxazole.

Therefore, trimetoprim/sulphametoxazole has an important role in the management of MRSA or MRCoNS infections. The MRCoNs showed very high resistance for trimetoprim/sulphametoxazole (86.7%) and erythromycin (96.7%). 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.

The study had demonstrated a high prevalence rate of MRSA with high rate of resistance to commonly used anti-staphylococcal agents. A large proportion of these MRSA and MRCoNS were found to be multi-drug resistant. There is need for continuous monitoring of antibiotic susceptibility pattern of all S. aureus isolates for selection of appropriate therapy. Also, infection control measures such as handwashing and other aseptic techniques must be followed to avoid therapeutic difficulties associated with these resistant pathogens. Further, molecular studies for studying and monitoring the epidemiology of MRSA and the multi-drug resistant MRSA in future is highly desirable. In developing countries, knowledge of antimicrobial resistance patterns is essential to define empirical therapy.

Table 1. Resistance and susceptible rate of MSSA, MRSA and MSCoNS, MRCoNS.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MSSA Resistance (%)</th>
<th>MSSA Susceptible (%)</th>
<th>MRSA Resistance (%)</th>
<th>MRSA Susceptible (%)</th>
<th>MRCoNS Resistance (%)</th>
<th>MRCoNS Susceptible (%)</th>
</tr>
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<tbody>
<tr>
<td>Penicillin</td>
<td>93.9</td>
<td>6.1</td>
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<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Rifampin</td>
<td>2.1</td>
<td>97.9</td>
<td>72.8</td>
<td>27.2</td>
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<td>100.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5.6</td>
<td>94.4</td>
<td>89.2</td>
<td>10.8</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>76.1</td>
<td>23.9</td>
<td>98.7</td>
<td>1.3</td>
<td>86.7</td>
<td>13.3</td>
</tr>
<tr>
<td>Trimetoprim/sulphametoxazole</td>
<td>1.9</td>
<td>98.1</td>
<td>19.0</td>
<td>81.0</td>
<td>29.6</td>
<td>70.4</td>
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<tr>
<td>Clindamycin</td>
<td>43.6</td>
<td>56.4</td>
<td>93.1</td>
<td>6.9</td>
<td>29.8</td>
<td>70.2</td>
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<tr>
<td>Vancomycin</td>
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<td>100.0</td>
<td>0</td>
<td>100.0</td>
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<tr>
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<td>0</td>
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<tr>
<td>Teicoplanin</td>
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<td>100.0</td>
<td>0</td>
<td>100.0</td>
<td>0</td>
<td>100.0</td>
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ACKNOWLEDGMENTS

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REFERENCES