Full Length Research Paper

Antimicrobial resistance pattern of MRSA strains isolated from patients of a hospital in Madinah, Kingdom of Saudi Arabia

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Methicillin-resistant Staphylococcus aureus (MRSA) strains are on the rise leading to severe morbidity and mortality in ICU patients worldwide including Saudi Arabia. In the present study, the prevalence, gender distribution and antimicrobial resistance pattern of this gram-positive nosocomial bacterial pathogen were explored. The strains were isolated from 6840 sampled patients at King Fahd Hospital, Madinah, KSA. Clinical sources were screened for MRSA strains during a period of 14 months and it was observed that MRSA represented only 5% of the total isolated positive samples. Exactly 237 MRSA strains were isolated from male patients and hence showed predominance over female samples, except in case of two sources (catheter tips and pus sample). Almost equal percentages of MRSA strains were recovered from wound swabs (124 strains) and sputum (120 strains) samples while 37 strains were recovered from nasal swabs. Collectively these three sources contributed majorly 84.7%. Antimicrobial sensitivity to conventional drugs was studied and the percentage resistance was in the following order: amoxicillin (99.5 %) > daptomycin, (98.8 %) > linezolid (98.0 %) > clindamycin (91.3 %) > erythromycin (90.8 %), > cotrimoxazole (84.4 %) > vancomycin (37.2 %). Vancomycin showed significant sensitivity of 62.8 % suggesting that this drug is a better option for effective treatment. After a high trend of MRSA infection (51%) during summer, a significant decrease (20.7 %) was observed during autumn which coincides with the annual Hajj season when special infection control provisions are taken. After Hajj season is over, infections decrease further to 18.5% during winter and 9.8% in spring. The present study is significant in being a step towards generation of national data on the prevalence of antimicrobial resistance patterns of MRSA.

Key words: Methicillin-resistant Staphylococcus aureus, gram positive bacteria, antimicrobial susceptibility pattern, antibiotic resistance, methicillin resistance.
INTRODUCTION

*Staphylococci* are non-spore forming Gram-positive bacteria that can persist in adverse environmental conditions. Although *Staphylococcus aureus* is a human commensal, it is a common source of skin infections, respiratory infections, and food poisoning in both community and hospital settings (Boswihi and Udo, 2018). In the last two decades, there has been an increase in infections caused by this human opportunistic pathogen particularly methicillin-resistant and vancomycin resistant *Staphylococcus aureus* strains (Shibli et al., 2013; McGuinness et al., 2017; Boswihi and Udo, 2018). Although vancomycin resistant species have been reported to be of low prevalence, methicillin resistance is more common in Saudi Arabia (Yezli et al., 2013; Tokajian, 2014). MRSA develops resistance to beta- lactam antibiotics including penicillin derivatives (such as methicillin, oxacillin), cephalosporins and also to macrolides, lincosides, and aminoglycosides (Boswihi and Udo, 2018). These strains are dangerous as they have bacterial genetic plasticity that allows them to acquire genetic materials that help in fighting antibiotics. The relatively high burden of MRSA is a major concern worldwide (Kapoor et al., 2017) as it leads to increased morbidity and mortality rates. Major risk factors that are associated with MRSA prevalence include diabetes, several other co-existing diseases, old age, misuse of antibiotics and prolonged stay in ICU (Graffunder and Venezia, 2002).

Several reports of MRSA emergence have been recorded from all over the world (Tong et al., 2015; Harkins et al., 2017; Udo and Boswihi, 2017). Since it harbors the multi-resistance genes, it has become difficult to treat with limited therapeutic options (Boswihi and Udo, 2018) posing serious public health issues. There are several reports from the Middle East. A study from Riyadh performed genotyping of MRSA strains and showed high diversity of clonal complexes (Monecke et al., 2012). Although systematic and regular screening of patients for MRSA is being carried out on regular basis, there has been increasing trend in the infection cases (Balkhy et al., 2007). According to previous studies the percentage of MRSA infection frequency in Saudi hospitals was around 40-50% of which more than 70% cases were resistant to erythromycin, gentamicin and oxytetracycline (Asghar and Momenah, 2006; Al Tayyar and Hawad, 2013). Another study from Riyadh claimed that for every 1000 admissions, 2 MRSA cases were reported (Al-Anazi, 2009). However, there are only a few reports from the two holy cities of Makkah and Madinah (Ali et al., 2013; Shirah et al., 2017).

Among gram-positive bacteria, MRSA is the most significant human pathogen and is a part of a larger problem called multi-drug resistance (MDR). This issue is increasing continuously at an alarming rate. As well defined information is not available about the prevalence and distribution of MRSA strains from Madinah. An attempt was made here to study clinical isolates from a busy hospital in this city of Saudi Arabia. In the current study we explore the prevalence, gender distribution and antimicrobial resistance pattern of MRSA strains isolated from patients at King Fahd Hospital.

MATERIALS AND METHODS

Sample collection

Different clinical samples (6840) mainly sputum, wound swab, nasal swab, tracheal aspirate, throat aspirate, catheter tip, pus, abdominal abscess, axilla, peritoneal wound swab, and semen were collected from patients suspected of bacterial infection at King Fahd Hospital at Madinah, Saudi Arabia. Clinical samples were cultured to isolate the organisms. Demographic data such as sex of the patients were recorded prior to sample collection.

Culture and Identification

The clinical samples were collected according to Center for Disease Control and Prevention Specimen Collection Guidelines (CDCP, 2013), aseptically inoculated on plates of blood agar, chocolate agar, Cystine-Lactose-Electrolyte-Deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) and incubated at 37°C for 24 h. Identification was done based on biochemical characteristics. Nasal swabs were also identified using BD GeneOhm™ MRSA ACP Lysis kit and BD GeneOhm™ MRSA Assay kit (BD diagnostics).

Biochemical characteristics

Suspected MRSA colonies isolated were further identified through Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.).

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents was determined by Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.). The following antimicrobial agents (obtained from BDH London, UK) were applied to each disc...
separately: linezolid (10 µg), daptomycin (30 µg), amoxicillin (20 µg), cotrimoxazole ([Trimethoprim-Sulfamethoxazole 1:19 (25 µg)]), vancomycin (50 µg), clindamycin (2 µg), and erythromycin (15 µg). The inoculum was prepared by growing various MRSA strains on separate agar plates. The colonies from the plates were transferred with a loop into 3 ml of normal saline. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of a Disc Dispenser (Oxoid Cambridge, UK), the discs were applied onto the surface of the inoculated agar and the plates were incubated overnight at 37°C. The diameter of the zone of inhibition observed was measured and compared to the chart provided by Clinical and Laboratory Standards Institute (CLSI, 2015).

RESULTS AND DISCUSSION

Samples (6840) were collected from several clinical sources for a period of 14 months and screened for MRSA strains. In comparison to other clinical isolates, only 5% MRSA strains were isolated (Figure 1). No strains were isolated from some sources such as bile, ascetic fluid, vaginal swab, urethral discharge, bone tissue, appendix, gall bladder aspirates, cystic fluid, necrotic tissue, pleural fluid, eye cornea swab, cerebrospinal fluid, ear, bed sores, and brain tube. As against other studies (Madani, 2002; Balkhy et al., 2007; Shibl et al., 2013) the MRSA isolation frequency, at 5% in the present study, was comparatively low. In one study (2004 to 2013) 6.5% MRSA strains were isolated from the sputum samples of pilgrims in Al Ansar hospital in Madinah (Shirah et al., 2017).

When samples were analyzed on the basis of gender, it was observed that around 71% strains were from male patients and 29% were from females (Figure 2). Retrieval of MRSA strains from different sources and their gender-wise distribution has been presented as Table 1. In wound swabs and sputum samples, 76.6 and 70% respectively were obtained from males. In case of nasal swabs also the isolates from male patients were greater than female patients at a percentage ratio of 64.9:35.1. The MRSA strains isolated from tracheal aspirate and throat aspirate were 6 and 10, respectively. Their gender-wise distribution was 5:1 and 9:1 respectively. The male to female ratio in axila and semen were 4:3 and 2:0, respectively. In case of abdominal abscess and peritoneal wound swab the isolates were low but male: female ratio was equal. In all cases the male samples predominated the female samples for MRSA isolation except in two cases. Interestingly, in the strains isolated from catheter tips and pus sample, female dominance could be seen at 53% isolation rates. The strains from pus samples were also greater from females but since the sample number was low the results may be insignificant.

The present study, in general, shows male predominance in MRSA infection and is in agreement with other previous studies (Ali et al., 2013). In one such study from Madinah (Shirah et al., 2017) it was found that three times more MRSA strains were isolated from male patients in all cases. A study from Makkah also reported a similar trend of higher MRSA isolation rates from males (Haseeb et al., 2016). This trend may be attributed to several probable reasons, from males being more prone to bacterial sepsis; being less compliant and hence predisposed to higher infection rates (Magliano et al., 2012). The female hormone, oestrogen, is reported to have an adverse effect on the expression of virulence
factors in pathogens (Neuman et al., 2015). Besides hand-hygiene behavior, life-style, occupation and socio-economic conditions of the patient play important roles in defining gender-infection relationships which of course need to be further explored (Humphreys et al., 2015). It is well known that the males constitute a bigger workforce in Saudi Arabia, and hence it is not surprising that samples obtained from male patients were greater in number.

The MRSA strains were isolated from a variety of clinical samples (Figure 3). Almost equal percentages of strains were recovered from wound swabs (37.4%) and sputum (36.1%) samples while 11.2% was recovered from nasal swabs. Collectively these three sources contributed the majority at 84.7%. High isolation rates from sputum and wound specimens have been reported earlier also (Masoud et al., 2011). Catheter tips, axilla, tracheal aspirate and abdominal abscess provided 5.1, 2.1, 1.8 and 1.2% respectively. From the remaining samples, that is pus, peritoneal wound swab and semen, the strain recovery was less than 1%.

Table 1 shows the result of antimicrobial susceptibility assay performed with seven antimicrobial drugs conventionally used against MRSA strains. Discs containing appropriate amounts as mentioned in materials and methods section were placed on Muller-Hinton agar and incubated at 37°C to see zone formation. All the drugs except vancomycin showed high resistance. The percentage resistance to the antimicrobials used in the present study was in the following order: amoxicillin (99.5%) > daptomycin, (98.8%) > linezolid (98.0%) > clindamycin (91.3%) > erythromycin (90.8%). > Cotrimoxazole (84.4%) > vancomycin (37.2%). Vancomycin showed appreciable sensitivity of 62.8 % against MRSA and hence proves to be an effective treatment option in comparison to other drugs. Previous studies from Saudi Arabia also show low vancomycin resistance among MRSA strains (Baddour et al., 2006; Alaklobi et al., 2015). As in our case, resistance to cotrimazol has been reported to be high (Yezli et al., 2013). Another study showed 100% resistance with linezolid (Al Tayyar and Hawad, 2013). Results corroborate previous data regarding MRSA resistance to drugs and present hope in vancomycin as a prescription drug against this gram positive bacteria. High resistance to other drugs suggests that physicians need to take utmost precaution while dealing with these drugs. There are reports from other places where strains developed resistance to vancomycin also (McGuinness et al., 2017; Al-Anazi, 2009; Yezli et al., 2013). Proper guidelines have to be followed given by reliable organizations such

Table 1. Gender wise distribution of MRSA strains isolated from different sources.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Sp</th>
<th>WS</th>
<th>NS</th>
<th>Tr</th>
<th>Th</th>
<th>Cath</th>
<th>Pus</th>
<th>Abd</th>
<th>Axilla</th>
<th>Peri</th>
<th>Semen</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>84(70)</td>
<td>95(76.6)</td>
<td>24(64.9)</td>
<td>5(83.3)</td>
<td>9(90)</td>
<td>8(47)</td>
<td>1(33.3)</td>
<td>2(50)</td>
<td>4(47.1)</td>
<td>1(50)</td>
<td>2(100)</td>
</tr>
<tr>
<td>F</td>
<td>36(30)</td>
<td>29(23.4)</td>
<td>13(35.1)</td>
<td>1(16.7)</td>
<td>1(10)</td>
<td>9(53)</td>
<td>2(66.7)</td>
<td>2(50)</td>
<td>3(42.9)</td>
<td>1(50)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>124</td>
<td>37</td>
<td>6</td>
<td>10</td>
<td>17</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

M, males; F, females; Sp, sputum; WS, wound swab; NS, nasal swab; Tr, tracheal aspirate; Th, throat aspirate; Cath, catheter tip; Abd, abdominal abscess; Peri, peritoneal wound swab. Percentage (%) values are given in parentheses.

Table 2. Antimicrobial susceptibility profile (%) of MRSA strains.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Resistant</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>2.0</td>
<td>98.0</td>
<td>0</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>1.2</td>
<td>98.8</td>
<td>0</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.5</td>
<td>99.5</td>
<td>0</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>15.6</td>
<td>84.4</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>62.8</td>
<td>37.2</td>
<td>0</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>8.7</td>
<td>91.3</td>
<td>0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>9.2</td>
<td>90.8</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3. Percentage of MRSA strains isolated from different clinical samples.
as the World Health Organization (WHO) and Gulf Cooperation Council (GCC) (Yezli et al., 2013). The implementation of their infection control and antimicrobial stewardship programs will definitely help in keeping the spread of resistant pathogens in control (Alawi and Darwesh, 2016; Zowawi, 2016; Alomi, 2017).

Since bacterial infections are significantly influenced by seasonal changes, the infection frequency for MRSA was also studied during the four major seasons in Madinah. As shown in Table 3, a high percentage of MRSA infection (51 %) was observed during summers, starting from 22nd June to 22nd September beyond which the percentage decreases till 21st December (20.7 %). This significant decrease during autumn is expected as this period coincides with the annual Hajj period when special efforts are taken to deal with all kinds of infections by the health authorities. As people from all over the world visit the two holy cities during this period, there are constant screening and prevention programs going on to avoid any kind of infection which otherwise may lead to uncontrollable epidemics (Alomi, 2017). During winters, after Hajj season is over, a further decrease (18.5 %) was observed. Spring exhibited an exceptionally low decrease here as frequency drops to 9.8 %. During spring season, the temperatures are not very high but as they rise during summers, there is a sudden surge in infections also. It is important to study these seasonal drifts which may be attributed to several factors (Fares, 2013). The data are to be corroborated by other studies on MRSA strains, isolated from patients in different other hospitals and time periods. Similar studies, along with improved infection control strategies will help in defeating multidrug resistance in both gram negative and gram positive bacteria.

Table 3. Percentage (%) of MRSA infection pattern during different seasons.

<table>
<thead>
<tr>
<th>Season</th>
<th>Percentage (%) of MRSA infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer (22 June -22 September)</td>
<td>51.0</td>
</tr>
<tr>
<td>Autumn (23 September-21 December): Pilgrimage season</td>
<td>20.7</td>
</tr>
<tr>
<td>Winter (22 December -30 March)</td>
<td>18.5</td>
</tr>
<tr>
<td>Spring (21 March-21 June)</td>
<td>9.8</td>
</tr>
</tbody>
</table>

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

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REFERENCES


