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PII: S1567-1348(15)30065-4
DOI: doi: 10.1016/j.meegid.2015.11.035
Reference: MEEGID 2568

To appear in:

Received date: 2 August 2015
Revised date: 2 November 2015
Accepted date: 30 November 2015

Please cite this article as: Hosseinkhani, Faride, Jabalameli, Fereshteh, Farahani, Narges Nodeh, Taherikalani, Morovat, van Leeuwen, Willem B., Emaneini, Mohammad, Variable number of tandem repeats profiles and antimicrobial resistance patterns of *Staphylococcus haemolyticus* strains isolated from blood cultures in children, (2015), doi: 10.1016/j.meegid.2015.11.035

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Variable number of tandem repeats profiles and antimicrobial resistance patterns of

*Staphylococcus haemolyticus* strains isolated from blood cultures in children

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ABSTRACT

*Staphylococcus haemolyticus* is a healthcare-associated pathogen and can cause a variety of lifethreatening infections. Additionally, multi-drug resistance (MDR), in particular methicillin-resistant *S. haemolyticus* (MRSH) isolates, have emerged. Dissemination of such strains can be of great concern in the hospital environment. A total number of 20 *S. haemolyticus* isolates from blood cultures obtained from children were included in this study. A high prevalence of MDR-MRSH isolates with high MIC values to vancomycin was found and 35% of the isolates were intermediate resistant to vancomycin. Multilocus variable number of tandem repeats analysis (MLVF) revealed 5 MLVF types among 20 isolates of *S. haemolyticus*. Twelve isolates shared the same MLVF type and were isolated from different wards in a pediatric hospital in Iran. This is a serious alarm for infection control; i.e. in the absence of adequate infection diagnostics and infection control guidelines, these resistant strains can spread to other sectors of a hospital and possibly among the community.

**Keywords:** *S. haemolyticus*, MLVF typing, multidrug resistance, blood culture, pediatric patients.
1. Introduction

*Staphylococcus haemolyticus* (SH) isolates have the ability to colonize the human skin and mucosal membranes (Cavanagh et al., 2012) and is the primary cause of catheter-related bloodstream infections (Flahaut et al., 2008; Takeuchi et al., 2005; Cavanagh et al., 2012). The ability of SH to develop multidrug-resistance (MDR), including to glycopeptides, represents a serious threat (Blavasco et al., 2000; Cavanagh et al., 2012; Flahaut et al., 2008). The genome of SH contains large amounts of mobile genetic elements coding for antimicrobial resistance, which can be transferred to related staphylococcal species (Vollú Silva et al., 2013). Colonized healthcare workers and medical devices may serve as a reservoir for transmission (Vollú Silva et al., 2013). Epidemiological analysis and identification of the antibacterial resistance pattern of this staphylococcal species are mandatory for appropriate infection control measurements (Hope et al., 2008).

Multilocus variable number of tandem repeats analysis (MLVF) is used to identify possible genetic associations among strains. MLVF is based on natural variation in the number of tandem DNA repeats, as present in multiple loci of most bacterial genomes and used as a phylogenetic marker (Cavanagh et al., 2012). This method is simple, rapid and highly discriminatory (Holmes et al., 2010).

In many microbiological diagnostic laboratories worldwide, SH is not identified at the species level. Hence, no informative data is available to address basic questions concerning its population structure (Becker et al., 2014).

The aim of this study was to evaluate the antimicrobial resistance of SH isolated from children's blood cultures and to identify possible transmission of isolates among patients in a pediatric hospital.
2. Materials and Methods

2.1. Strain collection

In total 20 SH strains isolated from blood cultures obtained from 18 children (10 children between 2-5 years, average 3.4 years and 8 newborns between 10 days through 11 months, average 99 days) hospitalized at Children's Medical Center Tehran, Iran during the period 2013 to 2014 were included. SH strains were obtained from patients hospitalized for more than 3 days (85%) and outpatients (15%).

Isolates were identified to the species level using standardized biochemical methods (Winn et al., 2006) and subsequently confirmed using a PCR-method targeting thermonuclease (nuc) gene as described previously (Hirotaki et al., 2011).

2.2. Antibiotic susceptibility testing

All isolates were tested for cefoxitin, ciprofloxacin, clindamycin, erythromycin, gatifloxacin, gentamicin, linezolid, rifampin, synergic (quinopristin-dalfopristin), tetracycline and trimethoprim/sulfamethoxazole using the disc agar diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2013, M100-S23). Minimum Inhibitory Concentration of all bacterial isolates to vancomycin and oxacillin was determined by the micro broth dilution method as recommended by CLSI (2013, M100-S23). S. aureus ATCC 29213 and Enterococcus faecalis ATCC29212 were used as reference strains. Multidrug-resistance was defined as resistance of the bacterial strain to three or more classes of antimicrobials (Cavanagh et al., 2012).

2.3. DNA extraction and mecA PCR

Template DNA was generated by boiling method as described previously (Fatholahzadeh et al., 2008). DNA was stored at −20 °C until use. Presence of the mecA gene was analysed for
all phenotypically oxacillin-resistant isolates using a PCR protocol as described previously (Blavasco et al., 2000).

2.4. MLVF typing

MLVF types were defined based on the difference in the 5 loci (L1-L5) and was performed as previously described. Isolates with a VNTR pattern difference in no more than one locus are considered the same type (Cavanagh et al., 2012).

3. Results

Antibiotic resistance patterns and molecular characterization of isolates are presented in Table 1. All isolates were susceptible to vancomycin, however 35% of the isolates demonstrated intermediate resistance (Table 2). The MIC$_{50}$ andMIC$_{90}$ of the isolates for vancomycin were 4mg/l and 8mg/l respectively. All strains were phenotypically oxacillin resistant (MIC$_{50}$ andMIC$_{90}$ values of 32mg/l and 128mg/l respectively). Only 80% of the isolates harbored the meca gene and were defined as MRSH isolates. Overall, 85% of the isolates were MDR.

Among the 5 loci studied in MLVF typing, no DNA fragment was observed in locus L5. Variable number of tandem repeats (VNTR) pattern analysis revealed 13 unique patterns and 5 different MLVF types among all SH isolates. The most predominant type (type I) included 12 isolates.

5. Discussion

Worldwide emergence of SH as a primary cause of infection has been reported (Becker et al., 2014; Gupta et al., 2012). Moreover, multidrug resistance has posed significant burden in the treatment of SH infections (Gupta et al., 2012). In this study, the frequency of MDR-SH isolates was high (85%), which is confirmed by reports from different parts of the world.
No *mecA* gene was detected in 20% of the phenotypically oxacillin-resistant SH isolates. Possible explanation may be the presence of a novel *mecA* allotype, beta-lactamase hyperproduction or alteration in genes encoding other penicillin binding proteins (Ba et al., 2014, Barros et al., 2012). Most of SH isolates were susceptible to rifampin and quinopristin-dalfopristin as reported previously (Becker et al., 2014). However, these antimicrobial agents are not recommended for treatment of blood infections in children or neonates according to their toxicity (Liu et al., 2011). In our study, resistance to tetracycline was significantly higher than mentioned in European and US studies (Coenen et al., 1997). This could be a possible effect of a more strict antibiotic policy in these countries (Becker et al., 2014). Although all isolates were susceptible to vancomycin, it is noteworthy to mention that the MIC level was high (MIC$_{50}$: 4mg/l, MIC$_{90}$: 8mg/l) and confirmed by previous studies from different part of the world (Barros et al., 2012; Becker et al., 2014; Vollú Silva et al., 2013). Latter phenomenon is a serious warning for infection control in the hospital environment. The extensive use of this antibiotic may cause the emergence of vancomycin-resistant strains (Emaneini et al., 2007; Kim et al., 2012; Kresken et al., 2009).

The high discriminatory power qualifies MLVF as a convenient typing method for the classification of SH isolates, which has a rather clonal population structure. High frequency of MLVF type I isolates and distribution of these isolates in the different wards of the hospital, indicating the presence of a common MDR clone in the hospital environment (Luczak-Kadlubowska et al., 2008).

In conclusion, this study revealed the high frequency of MDR-MRSH among different wards of an Iranian children’s hospital. In high-risk patients (neonates and immuno-compromised patients) infection with resistant clones can significantly increase the morbidity and mortality and a prolonged stay in the hospitals. (Cavanagh et al., 2012; Srinivasan et al., 2002; Forbes et al., 2007). This is a serious alarm for infection control; i.e. in the absence of proper and
suitable microbiological diagnostics and infection control guidelines, these resistant strains can spread to other sectors of a hospital and possibly among the community. Moreover, this species can act as a reservoir for the transmission of antibiotic resistance markers to other related and clinically relevant species, such as *S. aureus* (Becker et al., 2014).

**Conflict of interests**

The authors declare that there is no conflict of interest to reveal.

**Acknowledgments**

This project has been financially supported by Tehran University of Medical Sciences and Health Services, Tehran, Iran. Study grant no: 25170/93-02-30.

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Table 1. Antimicrobial resistance patterns and variable number of tandem repeats profile of *Staphylococcus haemolyticus* strains isolated from children’s blood cultures

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Ward**</th>
<th>VNTR(^1) loci (bp)</th>
<th>MLVF(^2) type</th>
<th>Resistance pattern</th>
<th>mecA</th>
<th>MDR(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EMR</td>
<td>500 500 700 100</td>
<td></td>
<td>ERY*, CLN, CIP, RIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>NICU</td>
<td>500 500 700 100</td>
<td></td>
<td>ERY, CLN, T, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>EMR</td>
<td>500 500 700 100</td>
<td></td>
<td>ERY, CIP, RIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>NICU</td>
<td>500 500 700 100</td>
<td></td>
<td>ERY, CLN, CIP, RIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>NEURO</td>
<td>500 500 100</td>
<td></td>
<td>ERY, CIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>INF</td>
<td>500 500 100</td>
<td>I</td>
<td>T, OXA ERY, CLN</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>EMR</td>
<td>500 500 400 100</td>
<td></td>
<td>TS, OXA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>NEPH</td>
<td>500 500 500 100</td>
<td></td>
<td>T, OXA ERY</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>OP</td>
<td>500 500 700 100</td>
<td></td>
<td>GM, OXA</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>EMR</td>
<td>500 - 700 100</td>
<td></td>
<td>ERY, CLN, SYN, CIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>EMR</td>
<td>500 300, 500 700 100</td>
<td></td>
<td>ERY, T, TS, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>EMR</td>
<td>500 300, 500 700 100</td>
<td></td>
<td>ERY, CLN, CIP, GAT, T, OXA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>SUR</td>
<td>500 300, 500 200 100</td>
<td>II</td>
<td>ERY, CLN, CIP, T, TS, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>OP</td>
<td>500 300, 500 200 100</td>
<td></td>
<td>ERY, CLN, OXA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>SUR</td>
<td>500 300, 500 200, 600 100</td>
<td></td>
<td>ERY, CLN, SYN, T, TS, OXA</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>NEURO</td>
<td>500 300, 500 400 100</td>
<td></td>
<td>ERY, RIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td>OP</td>
<td>500 300, 500 700 -</td>
<td>III</td>
<td>T, TS, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>18</td>
<td>NICU</td>
<td>500 300 200, 700 100</td>
<td>IV</td>
<td>ERY, CLN, CIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>INF</td>
<td>500 - 600 100</td>
<td></td>
<td>ERY, CIP, GAT, TS, GM, OXA</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>NEPH</td>
<td>500 - 600 100</td>
<td></td>
<td>ERY, CIP, GAT, TS, OXA</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Strains 5 and 16 were recovered from one patient and strains 13 and 15 from the other patient.

** EMR: emergency; NICU: neonatal intensive care unit; NEURO: neurology; INF: infant; NEPH: nephrology; OP: outpatient; SUR: surgery

\(^1\)VNTR: Variable Number of Tandem Repeat.

\(^2\)MLVF: Multi Locus Variable number of tandem repeats Fingerprinting

\(^*\)ERY, erythromycin; CLN, clindamycin; SYN, synercid; CIP, ciprofloxacin; RIP, rifampin; GAT, gatifloxac; T, Tetracycline; TS, trimethoprim-sulfamethoxazole; GM, gentamicin; OXA, oxacillin.

\(^\dagger\)MDR: Multidrug resistant
Table 2. Distribution of vancomycin and oxacillin MIC values among *Staphylococcus haemolyticus* strains isolated from children’s blood cultures.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Break point</th>
<th>≤0.5</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>16</th>
<th>32</th>
<th>64</th>
<th>≥128</th>
</tr>
</thead>
<tbody>
<tr>
<td>vancomycin</td>
<td>R* ≥32 µg/ml</td>
<td>4****</td>
<td></td>
<td>3</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>oxacillin</td>
<td>R ≥0.5 µg/ml</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Resistance, breakpoint is selected according to CLSI guidelines; ** MIC<sub>50</sub>: **; MIC<sub>90</sub>: Underlined

*** The number corresponds to the number of isolates with a certain MIC-value
Highlights

- High prevalence of MDR-\textit{Staphylococcus haemolyticus} was detected in blood cultures of children.
- 35\% of the isolates were intermediate resistant to vancomycin.
- All strains were susceptible to linezolid.
- 5 different types among 20 isolates of \textit{S. haemolyticus} were documented.
- Twelve isolates had same MLVF types and were isolated from different wards in a pediatric hospital of Iran.