Improved sensitivity for meticillin resistance detection in coagulase-negative staphylococci by moxalactam antibiotic discs or a cefoxitin investigation zone

Meticillin resistance in coagulase-negative staphylococci (CoNS) is very common and is usually mediated by mecA encoding penicillin-binding protein 2a (PBP2a; Fleer et al., 2012). Difficulties in the detection of meticillin resistance in CoNS are due to the variable phenotypic resistance expression of mecA, which is characterized by the presence of a small oxacillin-resistant subpopulation and/or transcriptional repression of mecA frequently found in CoNS (Dickinson & Archer, 2000; Finan et al., 2002; Nijjar et al., 2014). Therefore, the cefoxitin disc diffusion method, currently defined as a standard procedure by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), may fail to detect meticillin resistance in these isolates (CLSI, 2016; EUCAST, 2016). The Committee for Antibiotic Susceptibility Testing of the French Society of Microbiology (CA-SFM) introduced a 30 µg moxalactam disc in 2005, which has been proposed to be a better predictor than cefoxitin for the detection of meticillin resistance in CoNS (CA-SFM, 2012; Join-Lambert et al., 2007). We evaluated the effectiveness of various single and combined methods for the detection of meticillin resistance in CoNS, and derived a practical diagnostic approach.

A total of 103 non-duplicate clinical CoNS isolates were selected from 1581 clinical CoNS isolates obtained from 2013 to 2014 in the clinical laboratory of the Institute of Medical Microbiology, University of Zürich, Switzerland, as follows. All isolates with discrepant susceptibility categorization for moxalactam and cefoxitin according to EUCAST (2016)/CA-SFM (2012) guidelines were included (study group, n=18). Sixteen isolates were cefoxitin susceptible (≥25 mm) but moxalactam resistant (<23 mm), and two isolates were cefoxitin resistant (<25 mm) but moxalactam susceptible (≥24 mm). Eighty-five isolates that were uniformly categorized as susceptible (n=25) or resistant (n=60) to both cefoxitin and moxalactam, according to EUCAST (2016)/CA-SFM (2012) guidelines, were selected as a control group with a low/high likelihood for the absence/presence of mecA. Staphylococcus lugdunensis isolates were excluded from the analysis. CoNS species identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Biotyper, reference database version 3.1.2.0) comprised Staphylococcus epidermidis (n=86), Staphylococcus haemolyticus (n=6), Staphylococcus hominis (n=3), Staphylococcus pettenkoferi (n=2), Staphylococcus saprophyticus (n=5) and Staphylococcus warneri (n=1).

Disc diffusion was performed according to EUCAST recommendations. Inhibition zone diameters were determined using a Sirsca automatic zone reader (i2a). Susceptibility to 30 µg cefoxitin discs (i2a) was interpreted using the recommended current EUCAST/CLSI clinical breakpoints (CBPs) for CoNS, i.e. a susceptible CBP of ≥25 mm, and a resistant CBP of <25 mm (CLSI, 2016; EUCAST, 2016). For S. saprophyticus, EUCAST (2016) recommends that resistant/susceptible CBPs are <22 and ≥22 mm, respectively. Susceptibility categorization using the 30 µg moxalactam discs (i2a) was interpreted according to the disc diffusion CBP tables of the CA-SFM (resistant <23 mm, susceptible ≥24 mm) using guideline version 2012 (CBPs are no longer available in the 2015 version) (CA-SFM, 2012). Real-time PCR for mecA and mecC was performed on an ABI Fast TaqMan 7500 and is considered the gold standard for the presence of mecA/mecC (Pichon et al., 2012). Statistical significance was calculated with the Mann–Whitney U test using the spss statistics software version 20 (IBM Corporation).

Of the 103 isolates analysed, 81 (78.6 %) harboured mecA and 22 (21.4 %) were mecA negative (Table 1). mecC was not detected. Applying EUCAST/CLSI (2016) cefoxitin CBPs, CA-SFM (2012) cefoxitin CBPs, CA-SFM (2015) cefoxitin CBPs, and CA-SFM (2012) moxalactam CBPs, sensitivity for mecA detection was 74, 85, 79 and 93 %, respectively (Table 1). For the complete study population of 1581 isolates, sensitivity of CA-SFM (2012) cefoxitin CBP, CA-SFM (2015) cefoxitin CBP and CA-SFM (2012) moxalactam CBP was 98.9, 98.8 and 99.4 %, respectively. The overall specificity of cefoxitin disc diffusion CBPs for mecA detection ranged from 91 to 93 %, depending on the guidelines applied (Table 1).

We found a higher sensitivity for mecA detection applying moxalactam CA-SFM (2012) disc diffusion CBPs compared with cefoxitin CBPs of any guideline system examined. However, moxalactam CA-SFM (2012) CBPs are no longer contained in the current CA-SFM (2015) version, most probably to limit the complexity and size of drug panels. Cefoxitin disc diffusion testing results showed a lower sensitivity compared with other studies, most likely due to overrepresentation of critical/borderline isolates close to the CBP (Table 1) (Join-Lambert et al., 2007; Swenson et al., 2005, 2009). However, overrepresenting borderline and discrepant isolates allowed focusing on the comparison of moxalactam and cefoxitin CBP performance for critical isolates. Our results suggest that EUCAST/CLSI (2016)/CA-SFM (2015) cefoxitin CBPs may underestimate the presence of mecA among CoNS isolates, whereas CA-SFM (2012) cefoxitin CBPs had a higher sensitivity. Strictly following EUCAST (2016) guidelines, a significant number of AST (Antimicrobial Susceptibility Testing) reports for CoNS would result in ‘very major errors’ (categorization of true-resistant isolates as
PPV, positive predictive value; NPV, negative predictive value; TP, true positive; TN, true negative; FP, false positive; FN, false negative; S, susceptible; R, resistant; I, intermediate.

<table>
<thead>
<tr>
<th>Tests/criteria</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>TP (n)</th>
<th>TN (n)</th>
<th>FP (n)</th>
<th>FN (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin 30 μg disc, EUCAST/CLSI (2016)</td>
<td>74.1</td>
<td>90.9*</td>
<td>96.8*</td>
<td>48.8</td>
<td>60</td>
<td>20</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td>Cefoxitin 30 μg disc, CA-SFM (2012)</td>
<td>86.4</td>
<td>90.9*</td>
<td>97.2*</td>
<td>64.5</td>
<td>70</td>
<td>20</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Cefoxitin 30 μg disc, CA-SFM (2015)</td>
<td>85.2</td>
<td>63.6*</td>
<td>89.6*</td>
<td>53.8</td>
<td>69</td>
<td>14</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td>Moxalactam 30 μg disc, CA-SFM (2012)</td>
<td>92.6</td>
<td>95.5</td>
<td>98.7</td>
<td>77.8</td>
<td>75</td>
<td>21</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Moxalactam 30 μg disc, CA-SFM (2012)</td>
<td>92.6</td>
<td>86.4</td>
<td>96.2</td>
<td>76.0</td>
<td>75</td>
<td>19</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Moxalactam 30 μg disc, CA-SFM (2012)</td>
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Detection of mecA by PCR was considered the gold standard.

*If S. pettenkoferi was excluded from the analysis, the cefoxitin CBP specificities and PPV increased to 100%.

References


