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 Sircan automatic zone reader (2a). Susceptibility to 30 µg cefoxitin discs (2a) 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 (2a) 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).

The 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 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...
Detection of mecA with cefoxitin (Join-Lambert et al., 2007). EUCAST expert rules do not contain recommendations for meticillin resistance detection in CoNS, as do the guidelines of the CA-SFM, the BSAC (British Society of Antimicrobial Chemotherapy) and, partly, the CLSI guidelines (BSAC, 2014; CA-SFM, 2015; CLSI, 2016; EUCAST, 2011). Our results parallel those of other studies that found moxalactam to be a better predictor of meticillin resistance in CoNS compared with cefoxitin (Join-Lambert et al., 2007).

Using moxalactam discs alone or combining cefoxitin and moxalactam discs will improve sensitivity for mecA detection in CoNS compared with cefoxitin alone (Table 1). Alternatively, cefoxitin alone may be kept as the screening drug for CoNS, a diameter range (investigation zone) of ≥25 mm to ≤28 mm is proposed, indicating the need for confirmation testing such as PBP2a agglutination or mecA PCR. Of the 1581 CoNS isolates screened in this study, the number of isolates in this proposed cefoxitin investigation zone was 145 (9.2%). If PBP2a/mecA confirmation is limited to the clinically most important isolates, e.g. in intravenous catheter or prosthetic device-related infections, the number of isolates that have to be subjected to further investigation will decrease further. This approach would have resulted in a sensitivity and specificity of 96 and 95%, respectively.

The two moxalactam-susceptible but cefoxitin-resistant isolates were identified as S. pettenkoferi, a novel staphylococcal species, significantly different from all other Staphylococcus species, but closely related to the S. saprophyticus cluster and Staphylococcus auricularis (Fleet et al., 2012; Trulzsch et al., 2007). The moxalactam and cefoxitin zone diameters of the two isolates were 24 and 24 mm, and 21 and 24 mm, respectively. As the EUCAST CBP of S. saprophyticus is lower than that for other CoNS, further investigations may similarly lead to the re-evaluation of the CBP for S. pettenkoferi. If S. pettenkoferi was excluded from the analysis, the specificity of cefoxitin CBPs increased to 100% with all guideline systems (Table 1).

In summary, our results show: (i) the impact of an optimal combination of tests for mecA detection in CoNS; and (ii) the influence of CBP changes, which can significantly influence sensitivity, specificity, positive predictive value and negative predictive value of disc diffusion, and MIC critical values for the detection of meticillin resistance in CoNS.

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**References**


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### Table 1. Performance parameters of EUCAST, CLSI and CA-SFM cefoxitin and moxalactam disc diffusion CBPs, oxacillin MIC CBPs and PBP2a agglutination for the sample population studied (n=103)

<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><strong>Cefoxitin 30 µg disc, EUCAST/CLSI (2016)</strong> (CBP ≥ S/≤ R: 25/25 mm)</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><strong>Cefoxitin 30 µg disc, CA-SFM (2012)</strong> (CBP ≥ S/≤ I/R: 27/27 mm)</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><strong>Cefoxitin 30 µg disc, CA-SFM (2015)</strong> (CBP ≥ S/≤ I/R: 26/26 mm)</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><strong>Moxalactam 30 µg disc, CA-SFM (2012)</strong> (CBP ≥ S/≤ I/R: 24/24 mm)</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><strong>Moxalactam 30 µg disc, CA-SFM (2012)</strong> (CBP ≥ S/≤ I/R: 24/24 mm AND cefoxitin 30 µg disc EUCAST/CLSI (2016) (CBP ≥ S/≤ R: 25/25 mm))</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>
</tbody>
</table>

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%.


