P0820
Paper Poster Session
Phenotypic susceptibility testing of Gram-positive organisms

Are both oxacillin and cefoxitin testing necessary for the detection of methicillin resistance in coagulase-negative staphylococci?

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Background: The EUCAST breakpoint tables (2015) do not provide breakpoints for oxacillin against coagulase-negative staphylococci (CoNS) and the recommendation is to test cefoxitin. However, there is a comment indicating that the presence of the *mecA* gene correlates with an oxacillin MIC >0.25 mg/L for CoNS. In a nationwide prevalence study of *Staphylococcus* we observed that when using a commercial system for determining the cefoxitin MIC against CoNS, several *mecA*-positive isolates showed a cefoxitin MIC ≤ 4 mg/L (susceptible) but an oxacillin MIC of ≥0.5 mg/L. The objective of this study was to ascertain if both oxacillin and cefoxitin testing are necessary for the detection of meticillin-resistant (MR) CoNS.

Material/methods: In a nationwide prevalence study of *Staphylococcus* performed in Spain in 2014, we collected a total of 333 CoNS. The isolates were identified by MALDI-TOF. MICs of oxacillin and cefoxitin were tested by the automated broth microdilution method MicroScan (Beckman Coulter) following the manufacturer's guidelines, and the detection of the *mecA* gene was determined by PCR in all isolates. In addition, oxacillin and cefoxitin testing was performed by disk diffusion (DD) and by gradient diffusion (GD) in all *mecA*-positive isolates that showed a cefoxitin MIC of ≤4 mg/L (susceptible) or an oxacillin MIC of ≤0.25 mg/L by the automated method (AM). *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as control strains.

Results: Of the 333 CoNS, 169 (50.7%) were *mecA*-positive. Among the *mecA*-positive CoNS, 52 isolates (15%) were cefoxitin-susceptible by the MicroScan method (MIC ≤ 4 mg/L) after 24 h of incubation at 35-37ºC but showed oxacillin MICs of ≥0.5 mg/L. In addition, 3 *mecA*-positive isolates showed oxacillin MICs of <0.5 mg/L with the AM and were cefoxitin-resistant (Table). Among the 52 cefoxitin-susceptible isolates, 29 showed up as cefoxitin-resistant after 48 h of incubation with the AM. All *mecA*-positive isolates (n=52) were cefoxitin-resistant by the GD method, and 51 were cefoxitin-resistant by the DD method (one isolate showed a cefoxitin inhibition zone of 27 mm, susceptible). All isolates (n=52) were *Staphylococcus epidermidis*.

Conclusions: This study shows that when using the broth microdilution automated method (MicroScan), both oxacillin and cefoxitin testing are necessary for the appropriate detection of methicillin resistance in CoNS. Since many clinical laboratories use only broth microdilution automated methods routinely for the detection of methicillin-resistant staphylococci, we propose the inclusion of an oxacillin MIC breakpoint by the EUCAST committee.

CoNS isolates No. (%)	**Oxacillin MIC (mg/L)**	**Cefoxitin MIC (mg/L)**	**PCR *mecA***
163 (48.9)	≤0.25	≤4	Negative
3 (0.9)	≤0.25	>4	Positive
52 (15%)	0.5->2	≤4	Positive
1 (0.6%)	2	≤4	Negative
114 (34%)	>2	>4	Positive