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Paper Poster Session
New and old antibiotics against Gram-positive cocci in vitro

Ceftobiprole resistance in Danish MRSA

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Background: In 2013, ceftobiprole medocaril, the prodrug of the active moiety ceftobiprole, a novel cephalosporin with MRSA activity, was approved in European countries for the treatment of community-acquired pneumonia, and hospital-acquired pneumonia excluding ventilator-associated pneumonia.The frequency of resistance of MRSA to ceftobiprole is assumed very low, although studies in some African populations indicate higher resistance there. Resistance to ceftobiprole has been characterized as single non-synonymous mutations in the *mecA* gene causing conformational changes in the PBP2a protein. In 2013, ceftobiprole was implemented in the national surveillance of MRSA in Denmark including all new MRSA cases. In this study the level and mode of ceftobiprole resistance in Danish MRSA isolates were investigated.

Material/methods: MRSA isolates from January 2013 to March 2014, were tested for antimicrobial susceptibility with custom made TREK Sensititre panels (Thermo Fisher Scientific) for 21 antimicrobials including ceftobiprole (range 0.12-8 mg/L).

Confirmation of ceftobiprole resistance (EUCAST Clinical BP; MIC >2 mg/L) was performed by agar dilution MIC. Resistant isolates were investigated by whole genome sequencing, 2x 251 bp sequencing Nextera protocol, MiSeq (Illumina). Sequences were analysed in CLCbio software (Genomic Workbench, vers. 8.5) and aligned with reference MRSA genomes. Sequence types were obtained using the MLSTfinder (https://cge.cbs.dtu.dk/services/MLST-1.7/).

Results: A total of 3,505 MRSA isolates were tested and eight ceftobiprole resistant isolates were detected with MIC values in the range 4->=8 mg/L.

Subsequent MIC testing of the resistant isolates using agar dilution failed, however, to reproduce these findings and it was speculated that re-culturing of the isolates on non-selective blood agar plates had reverted the resistant geno- and pheno-types.

The isolates were therefore grown directly on selective agar plates containing 1 or 2 mg/L ceftobiprole and tested by agar dilution. Only two isolates reproducibly had MIC=2 mg/L, which is above EUCAST ECOFF MIC =1 mg/L. Analysis of the *mecA* gene in these two isolates revealed non-synonymous mutations resulting in aminoacid substitutions N204K, E239K and G246E in strain 88236 and N146K in strain 91992, respectively (Table 1). Analysis of *pbp*1-4, *gdpP* and *arcB* did not show any allelic variation that correlated to the observed resistance.

Table 1. *Characteristics of Ceftobiprole-Resistant MRSA in Denmark*

	MIC Ceftobiprole

# ID	MLST	*Spa*-type	*mecA*	PBP2a substitutions	TREK	Agar dilution
88236	ST22	t032	Positiv	N204K, E239K, G246E	4 mg/L	2 mg/L
91992	ST228	t041	Positiv	N146K	4 mg/L	2 mg/L

Conclusions: The study demonstrates very low resistance frequency to ceftobiprole (< 0.1%) in Danish MRSA. Alterations in the allosteric domain of PBP2a in the two resistant isolates were likely responsible for the observed resistance, as they have previously been described to cause ceftaroline resistance. The detection of ceftobiprole resistance is complicated by the observation that resistant mutants may revert easily under non-selective.