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BOOK OF ABSTRACTS

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P118. Identification of 2CS-CHXT operon signature of chlorhexidine tolerance among *Enterococcus faecium*

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Background: Chlorhexidine-gluconate (CHX) activity against *Enterococcus faecium*-*Efm* is scarcely documented, with most available data not addressing the clonal background of the strains (clades A1-infection derived strains, A2-mostly animals, B-human commensal). A P102H-mutation in a conserved DNA-binding-response-regulator (ChtR) has been associated with chlorhexidine tolerance among strains of *Efm* clade A1, although the operon remained unidentified (PMID:28242664). Here, we evaluated CHX activity, the distribution of ChtR-P102H, the predicted ChtR operon and its variability among *Efm* from diverse sources and clades.

Methods: *Efm* (n=106) from clades A1 (n=48; human/animal/food/environment), A2 (n=43; human/animal/food) and B (n=15; human/animal/environment) (1995-2016; 5-countries; multidrug-resistant:72%) were included. CHX susceptibility (range:2-32mg/L) was determined by broth- microdilution. *Efm* MIC distribution was analysed by ECOFFINDER-tool (http://www.eucast.org/mic_distributions_and_ecoffs/). Thirty-seven *Efm* were sequenced (Illumina-NextSeq platform/2X150bp paired-end). DOOR-2.0 operon database (<http://csbl.bmb.uga.edu/DOOR/index.php>) predicted ChtR operon. Amino-acid mutations in ChtR and other operon proteins were identified by comparison (BLASTp-NCBI) with the CHX-tolerant reference strain ChtR-P102H-*Efm*-E1162 (EFF34003.1; PMID:28242664).

Results: CHX-MIC ranged between ≤ 2 -32mg/L, with the MICs fitted curve slightly deviated to the left comparing to raw data distribution, suggesting the presence of a non-wild-*Efm* population. Most *Efm* with MIC ≥ 8 mg/L (89%-n=25/28; 3 clades; 54% of clade A1) presented the ChtR-P102H, while most isolates with MIC ≤ 4 mg/L did not (89%-n=8/9; clades A2/B). The predicted 4086bp-operon associated with *chtR* included a previously identified sensor-histidine-kinase as well as a genes coding for proteins related to a glucose:proton symporter and an amino acid permease of the Amino acid-Polyamine- organoCation (APC) family, firstly described here. The complete operon was present in all 37 *Efm* sequenced. Most of 28 *Efm*-MIC ≥ 8 mg/L exhibited operon sequences identical to ChtR-P102H-*Efm*- E1162, contrasting with diverse amino-acid mutations identified in the sensor-histidine-kinase and/or in the two new transporters proteins identified in isolates with a CHX-MIC ≤ 4 mg/L and lacking ChtR- P102H.

Conclusions: The complete characterization of the ChtR-P102H-operon, highly conserved among *Efm* with high CHX-MICs, is here firstly described. The ChtR-P102H mutation associated with CHX tolerance is spread in *Efm* from different sources and clades, but mostly from clade A1. The role of each ChtR-operon protein in the CHX-tolerance as well as the occurrence of other CHX tolerance mechanisms in isolates with MIC ≥ 8 mg/L and lacking ChtR-P102H deserves further research.