29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

P2334 Identification of a conserved operon in chlorhexidine tolerant *Enterococcus faecium* from different clades and origins

Barbara Duarte[1], Ana P. Pereira[2], Ana Raquel Freitas[1], Teresa M. Coque Gonzalez[3,4], Henrik Hasman[5], Anette M. Hammerum[5], Patricia Antunes[1,6], Luisa Maria Vieira Peixe[1], Carla Novais*[1]

[1] UCIBIO/REQUIMTE. Faculty of Pharmacy. Biological Sciences Department., University of Porto, Porto, Portugal, [2] Faculty of Pharmacy. Biological Sciences Department., University of Porto, Porto, Portugal, [3] University Hospital Ramón y Cajal, Madrid, Spain, [4] CIBER Epidemiología y Salud Pública, Madrid, Spain, [5] Statens Serum Institut. Department of Microbiology and Infection Control, Copenhagen, Denmark, [6] Faculty of Food Sciences and Nutrition, University of Porto, Porto, Portugal

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 regulon remained unidentified (PMID:28242664). Here, we evaluated CHX activity, the distribution of ChtR-P102H, the predicted ChtR regulon and its variability among *Efm* from diverse sources and clades.

Materials/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-five Efm were sequenced (Illumina-NextSeq platform/2X150bp paired-end). DOOR software (http://csbl.bmb.uga.edu/DOOR/index.php) predicted ChtR regulon. 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 (mode=16mg/L; 51% of isolates), 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 of Efm with a MIC≥8mg/L (96%-n=25/26; 3 clades; 14 of infections) presented the ChtR-P102H, while most isolates with a MIC≤4mg/L did not (67%-n=6/9; clades A2/B; 2 of infections). The predicted 4101bp-operon associated with *chtR* included a previously identified sensor-histidine-kinase as well as a DMT superfamily drug/metabolite transporter and an amino-acid-polyamine-organocation family transporter genes, firstly described here. The complete operon was present in all 35 Efm sequenced. The 25 Efm-MIC≥8mg/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≤4mg/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. Its wide distribution in Efm of diverse clades and sources suggests occurrence of horizontal transfer events. 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≥8mg/L and lacking ChtR-P102H deserves further research.