Hospital wastewater as a route for transmission carbapenem-resistant *Acinetobacter baumannii* outside hospital setting

Ivana Goic-Barisic[1], Ana Kovacic[2], Marija Tonkic[1], Martina Seruga-Music[3], Anita Novak[1], Zana Rubic[1], Jasna Hrenovic[3]
[1]University Hospital Centre Split and School of Medicine University of Split, Split, Croatia
[2]Institute of Public Health of Split and Dalmatia Country, Split, Croatia
[3]Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia

27th ECCMID Vienna, Austria 22 – 25 April 2017
The congress of ESCMID

eP 2254

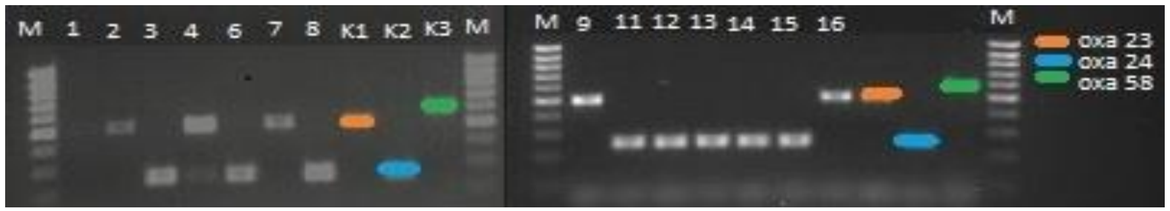
Background

Acinetobacter baumannii origin and epidemiology is under a great concern worldwide since this microorganism has become a leading nosocomial pathogen of the 21th century among the "ESCAPE" group of microorganisms. Since 2009 University Hospital Centre Split (UHCS) in Croatia has a growing problem in the number of infections caused by carbapenem-resistant isolates of *A. baumannii* which is now almost endemically present in most of the intensive care units inside the hospital. The recent literature confirmed the appearance of carbapenem resistant isolates of *A. baumannii* in nature that correlated with clinical isolates. Therefore, in order to explore epidemiology and surveillance control of this important hospital pathogen in Croatia we investigated presence of *A. baumannii* in hospital wastewater as a route for possible transmission outside of hospital setting.

Materials/methods

For prospective investigation UHCS wastewater was sampled for five times, on two different locations, in the period from October 2015 to April 2016. Samples of hospital wastewater were taken in 500 ml sterile bottles and inoculated within two hours on solid media. The isolation of *A. baumannii* was performed on CHROMagar Acinetobacter supplemented with CR102 (CHROMagar) and 15mg/L of cefsulodin sodium salt hydrate (Sigma-Aldrich). The plates were incubated at 42°C/48h. Identification of *A. baumannii* was performed by routine bacteriological techniques and confirmed by MALDI-TOF MS (Bruker Daltonics) on cell extracts. Antibiotic susceptibility was assessed by disk diffusion method. The MICs values were confirmed by Vitek2 system or E-tests (AB Biodisk), and interpreted according to the EUCAST criteria, except for ampicillin/sulbactam and tigecycline that were interpreted according CLSI criteria. The presence of *bla*OXA genes encoding OXA-type carbapenemases (OXA-51-like, OXA-23like, OXA-40-like, OXA-58-like, and OXA-143) was investigated by multiplex PCR and sequencing. Genotyping was performed using PFGE analysis and the results were compared with unpublished data of previously typed four clinical isolates (c.i.) from the same monitoring period.

Fig.1 Multiplex PCR results from wastewater isolates (2-12) and clinical isolates (13-16). 1-neg control. K 1-3 pos control



Results

During the examination period fourteen both carbapenem and multi-resistant isolates of *A. baumannii* were isolated from hospital wastewater. According to the PFGE analysis and resistance phenotype (profile) 9 isolates (2-4, 6-9 and 11-12) were selected for further molecular characterization and comparison with four clinical isolates. The clinical isolates were collected in the same period of time, during routine surveillance of patient's samples (tracheal aspirates).

Multiplex PCR confirmed that wastewater isolates 2,4,7 and 9 harbored *bla*OXA-23-like while wastewater isolates 3,6,8,11 and 12 harbored *bla*OXA-40-like genes (Fig 1). Phylogenetic analyses of all amplified and sequenced *bla*OXA fragments clearly supported the affiliation of detected *bla*OXA genes to two different clusters identical as those from clinical isolates (13-16) and available in GenBank (Fig 2). Clinical isolates 13-15 shared 100% sequence identity with *bla*OXA-72 sequence described in the same hospital in 2009, confirming the presence of endemic cluster. Since OXA-72 within OXA-40-like group was described as dominant mechanism of resistance in clinical isolates of *A. baumannii* in 2009 inside UHCS, this investigation revealed also a new oxacillinase belonging to OXA-23-like group (c.i.16) which contributed to the resistance rate to carbapenems of 90% in the last two years.

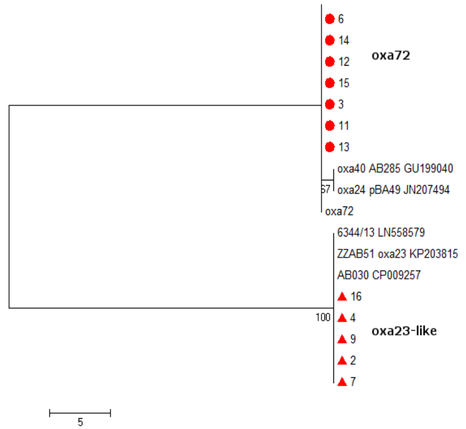


Fig.2 Phylogenetic tree constructed on the basis of *bla*OXA genes encoding OXA-type carbapenemases for wastewater isolates (2-12) and clinical isolates from UHCS (13-16). GenBank accession numbers are given next to the name of each strain.

Conclusion

This study confirmed the possible spread of multi-resistant *A. baumannii* through hospital wastewater in nature. The possible impact on the horizontal transfer of *bla*OXA genes, surviving in selected condition or occurrence of infection outside the hospital setting should be further investigated.

This research was supported by University of Split School of Medicine and a part by the Croatian Science Foundation (grant no. IP-2014-09-5656).

University of Split
School of Medicine
Universitas Studiorum
Spalatensis
Facultas Medica