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Main topic : Antimicrobial resistance: A worldwide concern

Presence of clinically relevant carbapenemase-producing *Klebsiella pneumoniae* in wastewater in Belgrade, Serbia

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Introduction: To fight antimicrobial resistance (AMR), it is important to support a "One Health" approach (human, animal, and environmental health). The wastewater (WW) is considered a source of clinically relevant antibiotic-resistant bacteria and may thus be important for their dissemination into the environment, especially in countries with poor WW treatment. To get an overview of the occurrence and characteristics of carbapenemase-producing *Klebsiella pneumoniae*, critical multidrug-resistant pathogen according to WHO priority list, in WW of Belgrade, we investigated samples from four main sewer outlets prior to effluent into international rivers, the Sava and the Danube.

Methods: Samples of WW were collected from four main sewer outlets between May and August 2018. For each water sample, 50 mL was inoculated into 50 mL of enrichment broth. After overnight incubation, screening for target bacteria was performed by streaking one loopful of enriched culture onto CHROMID® CARBA SMART (bioMérieux, Marcy-l'Étoile, France). The isolates were identified by MALDI-TOF MS (VITEK® MS, bioMérieux). Whole-genome sequencing was performed on a MiSeq® Platform (Illumina) and sequencing data were analysed with the easy-to-use, fully integrated web-based software application EPISEQ® CS (bioMérieux) for genomic characterisation and wgMLST-based epidemiological analysis.

Results: Overall, 15 WW samples were collected and investigated within this study and 12 target *K. pneumoniae* isolates were analysed. The *K. pneumoniae* isolates resolved into six sequence types (STs), of which one isolate presented new ST (ST6273). The most frequent was ST101 (n=7, 58.3%), whereas other STs represented singletons: ST15, ST16, ST29, ST437 and ST6273. The predominant ESBL gene in all *K. pneumoniae* isolates was blaCTX-M-15, followed by blaOXA-1 (83.3%). All isolates harboured carbapenemase blaOXA-48 gene, with one isolate co-harboring blaOXA-48 and blaNDM-1. Regarding the resistance to colistin, 85.7% ST101 *K. pneumoniae* isolates had missense mutation (TGC->AGC) in mgrB gene leading to substitution C28S in the MgrB regulator, and in one isolate mutation in the basS/basR two-component system was detected. The majority of *K. pneumoniae* isolates (91.7%) had four or more different plasmid replicon types. The most common was Col440II (75%), followed by IncFIA(HI1) (58.3%), IncR (58.3%), IncL (50%), IncFIB (pKPHS1) (50%) and others. Finally, among ST101 *K. pneumoniae* two clusters of closely related isolates were detected (99.55% similarity), sharing the same resistome and plasmid replicons but not strictly the same virulome. Cluster 1 grouped isolates (n=2) from the same sampling site (S2, sewer outlet with no hospital input), but from different sampling campaigns (SC1-May and SC4-August), indicating the persistence of this clone over time. Cluster 2 included isolates (n=3) from the same sampling campaign (SC3-July), two from sampling site S1 (input from multiple hospitals) and one from site S3 (input from single tertiary healthcare hospital) located downstream of S1, indicating probable spread to the ecosystem.

Conclusions: This study showed the presence of carbapenemase-producing colistin resistant *K. pneumoniae* in wastewater in Belgrade, Serbia, and highlighted a possible role of aquatic environment as a reservoir of clinically relevant antibiotic-resistant bacteria, with potential to spread throughout the community and serious threat on human and animal health.