

TITLE: FROM THE HOSPITAL EFFLUENT TO THE URBAN WASTEWATER TREATMENT PLANT: COPRODUCTION OF KPC, ESBL AND RmtG GENES IN *Klebsiella pneumoniae* AND *Enterobacter cloacae*

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ABSTRACT

Hospital effluents are reservoirs of multidrug-resistant bacteria (MRB) with several acquired antibiotic resistance determinants (ADR) due to its extremely high selective pressure of antimicrobials. To evaluate the route of dissemination of MRB in the effluents, we analyzed Enterobacteriaceae recovered from sewage samples from the hospital to the urban wastewater treatment plant of Londrina, Brazil. Samples of effluents were collected in duplicate at each location: final effluent from the University Hospital of Londrina (EUHL), Elevation Sewage Station of Limoeiro (ESSL), Municipal Wastewater Treatment Plant before treatment (MW-BT) and after treatment (MW-AT) in november of 2012 and february of 2013. The identification and the antimicrobial susceptibility tests of the isolates were performed by conventional biochemical tests and automatized system Vitek-2, for 9 KPC-producers isolates. The presence of ADR was investigated by PCR and the clonal relatedness of the isolates were conducted by ERIC-PCR with a 93% cutoff. Of total of 138 Gram-negative bacilli identified as being Enterobacteriaceae, 9 (6,5%) bacterial isolates of *Klebsiella pneumoniae* (n=6) and of *Enterobacter cloacae* (n=3), recovered from EUHL (n=6), MW-AT (n=2), and ESSL (n=1), showed the *bla*_{KPC} gene. Besides *bla*_{KPC}, co-production of other genes codifying β -lactamases was founded in the isolates, including: *bla*_{CTX-M} (n=7), *bla*_{TEM} (n=6), and *bla*_{SHV} (n=3). Additionally, it was detected several *bla*_{CTX-M} groups including: CTX-M-2 (n=3), CTX-M-9 (n=2), CTX-M-1 (n=1), and CTX-M-8 (n=1). Only 2 isolates of *K. pneumoniae* recovered from UHL and MW-AT were positive for RmtG. All the isolates were negative for metallo-beta-lactamases and *qnr* genes. A genetic diversity pattern was detected, in which 7 clonal lineages were verified (A-G). Surprisingly, it was detected that two isolates belonging to the clone D of *K. pneumoniae* that harboring *bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M-2}, *bla*_{KPC}, and RmtG genes were isolated from UHL and MW-AT. These findings are worrisome once a dissemination pathway of highly contaminated MRB carrying important ADR between the UHL effluent and the MW-AT was demonstrated, suggesting the inefficacy of an effective treatment to remove MRB of the wastewater network and reinforcing the hospital role in the discharge of these pathogens in the environment. Hence, improvements in hospital effluents as well as wastewater treatment process are essential in order to protect public health.

Keywords: Antibiotic resistance, β -lactamases, Enterobacteriaceae, hospital effluent, wastewater treatment plant

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