

TITLE: RETROSPECTIVE STUDY OF 3RD-4TH GENERATION CEPHALOSPORINS-RESISTANT *ENTEROBACTER CLOACAE* COMPLEX ISOLATES IN AN UNIVERSITY HOSPITAL: PREVALENCE OF *ENTEROBACTER HORMAECHEI*

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ABSTRACT:

Enterobacter cloacae Complex (EcC) species have been isolated among top five Gram-negative bacilli in hospital infections, highlighting carbapenem-resistant and 3rd generation cephalosporin-resistant isolates, recognized priority one pathogens (critical *Enterobacteriaceae*) according to World Health Organization. The objective of this study was to identify EcC species, to determine the population structure, and searching for extended-spectrum beta-lactamases (ESBL) and AmpC-overproduction in EcC isolates resistant to 3rd-4th generation cephalosporins isolated in an university hospital in Brazil. EcC species (n=24) were isolated during 7 months in 2007. Species assignment was performed by *hsp60* gene sequencing. Clonality was assessed using *Xba*I-pulsed-field gel electrophoresis and the results were analyzed using the Tenover criteria. ESBL production was screened using double disc synergy test (DDST, clavulanate inhibitor) and AmpC-overproduction was detected using combined disk test (cloxacillin inhibitor), both tests evaluating cefotaxime, ceftazidime, cefepime and aztreonam. Genes coding for ESBLs (*bla*_{CTX-M}, *TEM*, *SHV*) were searched by PCR and sequencing. Among the 24 isolates of the EcC, 22 (92%, 22 different pulsotypes) were identified as *Enterobacter hormaechei*, 1 (4%) as *Enterobacter asburiae* and 1 (4%) as *Enterobacter cloacae*. Beyond cross-resistance to extended-spectrum cephalosporins and aztreonam, co-resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulphamethoxazole were frequently detected. MDR phenotype was determined for all isolates studied, except for *E. asburiae*. ESBL production was found in 9/24 EcC isolates, including the single *E. cloacae*. AmpC-overproduction was detected in 12/24 EcC isolates, including the single *E. asburiae*. ESBL production associated to AmpC-overproduction was detected in 3 EcC isolates. ESBL-producing *E. hormaechei* isolates harbored *bla*_{CTX-M-9} (n=4), *bla*_{SHV-5} (n=7) or *bla*_{CTX-M-2} (n=1). The single ESBL-producing *E. cloacae* harbored *bla*_{CTX-M-9}. In 2007, *E. hormaechei* was the prevalent EcC species, showing high clonal diversity. Moreover, there was similar prevalence of acquired and chromosomal beta-lactamase genes. Retrospective studies are important to know the prevalence of EcC species and resistance mechanisms, contributing to understand the evolution of antimicrobial resistance in EcC species hospital infections.

Keywords: *bla*_{CTX-M}, *bla*_{SHV}, ESBL, AmpC, resistance genes

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