TITLE: ENTEROBACTER HORMAECHIEI AS PREVALENT SPECIES OF ENTEROBACTER CLOACAE COMPLEX IN A UNIVERSITY HOSPITAL: HIGHLIGHTING MULTICLONALITY, MULTIDRUG-RESISTANCE AND BROAD PRESENCE OF HEAVY METAL TOLERANCE GENES


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ABSTRACT:

Enterobacter cloacae Complex (EcC) species have been isolated among top five Gram-negative bacteria from hospital infection. The objectives of this study were to identify EcC species, to determine population structure and to investigate acquired beta-lactamases and heavy metal tolerance genes of isolates from two different periods. Carbapenem-resistant and/or 3rd-4th generation cephalosporin-resistant EcC isolates from 2007 (n=24) and 2016 (n=28) were studied. Species assignment was performed by hsp60 partial gene sequencing. Clonality was assessed using XbaI-pulsed-field gel electrophoresis. Genes coding for extended-spectrum beta-lactamase (blaCTX-M, TEM, SHV) and carbapenemases (blaIMP, VIM, NDM, OXA-48, KPC) were investigated by PCR and sequencing. Moreover, genes coding for acquired heavy metal tolerance to silver (silA), copper (pcoD), arsenite (arsB), tellurite (terF) and mercuric (merA) were detected by PCR. Among EcC from 2007: 22 (92%) were identified as Enterobacter hormaechei, all different pulsotypes; and Enterobacter asburiae and Enterobacter cloacae 1 (4%) of each. blaCTX-M-(group 9) (n=5), blaCTX-M-(group 2) (n=1) and blashv (n=4); silA (n=11), pcoD (n=9), terF (n=13), arsB (n=2), merA (n=9), silA/pcoD (n=9) and silA/pcoD/merA (n=6) were found. However, only one E. hormaechei and the single E. cloacae, both CTX-M producers, harbored all heavy metal tolerance genes searched. Among EcC from 2016: 27 (96%) were identified as E. hormaechei, pulsotype “A” (n=6) and the last ones different pulsotypes; and 1 (4%) as Enterobacter kobei. blaKPC (n=28), blaCTX-M (group 1) (n=26) and blatem (n=26); silA (n=21), pcoD (n=23), arsB (n=22), terF (n=26), merA (n=11), silA/pcoD/terF (n=21) and silA/pcoD/terF/arsB (n=17) were found. Highlighted, 10 (37%) E. hormaechei carried all heavy metal tolerance genes searched. Overall, E. hormaechei species was prevalent in both periods evaluated (2007 and 2016), displaying multiclonal and multidrug-resistance phenotype, however, only isolates from 2016 were KPC producers. Probably, blaKPC (blaCTX-M-group 1 and/or blatem) was harbored on highly transmissible plasmid circulating among EcC isolates. Moreover, there was highlighted broad presence (and different sets) of heavy metal tolerance genes in EcC isolated in 2016, thereby, representing concern due to possibility of co-selection in the presence of antibiotic or heavy metal in the bacteria from hospital studied.

Keywords: antibiotic resistance, beta-lactams, beta-lactamase, heavy metal tolerance, hospital infection.