TITLE: SUBPOPULATIONS OF NDM-1 PRODUCING *Enterobacter hormaechei* CLINICAL ISOLATE SHOW DIFFERENCES IN ANTIMICROBIAL TREATMENT RESPONSE AND IN BIOFILM FORMATION

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ABSTRACT

Enterobacter spp. have increasingly been recognized as a nosocomial pathogen representing the third major Enterobacteriaceae species involved with infections and the second most prevalent carbapenemase producing genus. The aim of this study was to grow macrocolony of *Enterobacter hormaechei* subsp. *oharae* clinical isolates harboring bla_{NDM-1} gene (New Delhi metallo-beta-lactamase) and to evaluate virulence mechanisms and antimicrobial susceptibility of different subpopulations generated from the macrocolony. Nine *E. hormaechei* clinical isolates were cultured during five days to promote the growth of macrocolony biofilms. Subpopulations generated by the macrocolony of one clinical isolate were used for further experiments. Antimicrobial susceptibility was determined by minimum inhibitory concentration and synergistic effect of double and triple combination was evaluated by checkerboard. The in vivo *Galleria mellonella* larvae model was used to analyze the virulence of NDM-1 producing *E. hormaechei* and the efficacy of antimicrobial treatments. This study shows that subpopulations from the same macrocolony can produce different amount of biofilm and type 3 fimbriae seems to be straightly related with biofilm forming ability. Regarding antimicrobial susceptibility, discrepancies between *in vitro* and *in vivo* results were observed. *In vitro*, triple combination with meropenem-rifampicin-polymyxin B showed synergistic effect against all tested subpopulations. While, *in vivo*, using *G. mellonella* larvae model, triple combination increased larvae survival infected with 798-4S but was not effective to control infection with 798-1S. We also observed that these subpopulations showed different response to meropenem treatment in *G. mellonella*. The ability of multidrug resistant *E. hormaechei* in generating bacterial subpopulations presenting different susceptible profile and virulence mechanisms is worrisome and may explain why these infections are hardly overcome.

Keywords: *Enterobacter*, biofilm macrocolony, antimicrobial resistance, *Galleria mellonella*

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