Poster

Synergic effect of oxyclozanide in combination with colistin against colistin-resistant and colistin-susceptible clinical strains of Klebsiella pneumoniae .

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ABSTRACT

Motivation: Colistin is among the few antibiotics effective against Klebsiella pneumoniae clinical isolates. However, in the last few years, colistin-resistant K. pneumoniae have been isolated (1). Therefore, combination therapies between colistin and old drug effective against these isolates are required. The objective of this study is to study in vitro the activity of oxyclozanide, an anthelmintic drug (2), in combination with colistin against colistin-susceptible (Col-S) and colistin-resistant K. pneumoniae.

Methods: Col-R (KPc21) and Col-S (CECT 997) K. pneumonia strains were used. Checkerboard assay with colistin and oxyclozanide to study the synergy between both drugs was performed. Time-kill assays using both strains at 6 log CFU/ml, colistin and oxyclozanide were tested alone and in combination with sub-minimal inhibitory concentration (MIC) of colistin (0.25 µg/ml for CECT 997 strain and 16 µg/ml for KPc21 strain) and oxyclozanide at 2 µg/ml. Analysis of KPc21 and CECT 997 strains cell walls in presence of 2 µg/ml oxyclozanide during 24 h by transmission electron microscopy (TEM) was performed. Permeability assays and outer membrane proteins (OMPs) profile analysis by SDS-PAGE of both strains were performed.

Results: Checkerboard assay showed a synergic effect between colistin and oxyclozanide against the KPc21 strain (Fold change = 8), but not for CECT 997 strain (Fold change = 2). Time-kill assays showed a synergic effect between colistin and oxyclozanide against the KPc21 strain (decreasing the bacterial growth by 3.24 log CFU/mL) at 24 h, but not against the CECT 997 strain whose bacterial growth was reduced by 0.45 log CFU/mL. Incubation with oxyclozanide at 24 h did not cause change on the OMPs profile of both strains. Futhermore, the images from TEM showed that oxyclozanide disrupted the bacterial cell envelope affecting its permeability. The membrane permeabilization assay confirmed these data, in which the Col-R strain had higher membrane permeability.

Conclusions: From these in vitro data, we concluded that oxyclozanide potentiates the bactericidal activity of colistin by disrupting the bacterial cell envelope. For this reason, oxyclozanide would be a good adjuvant for colistin to treating the infections caused by K. pneumoniae.

REFERENCES

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