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Poster Session IV
Enterobacteriaceae: resistance and fitness

BETA-LACTAMASE-PRODUCING GRAM NEGATIVE ISOLATES AND MOLECULAR MECHANISMS INVOLVED IN THE EXPRESSION OF TIGECYCLINE RESISTANCE

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Objectives:

The emergence of antibiotic resistant Gram negative bacteria, particularly β-lactamase-producing bacteria, seriously compromises the efficacy of the treatment options available. This study aimed to evaluate the antimicrobial susceptibility, and to characterize the tigecycline resistance due to efflux pump production, in Gram negative clinical isolates. Moreover, it meant to clarify the *in vitro* activity of tigecycline against isolates resistant to other antibiotic classes, such as β-lactams, eventually related to β-lactamase production.

Material and Methods:

A total of 211 isolates collected in 2010 and 2012, were studied. The antimicrobial susceptibility of all isolates was performed by disc diffusion method and minimal inhibitory concentration (MIC), and interpreted by the SFM guidelines. The antibiotics tested were the following: colistin, ciprofloxacin, imipinem, cefoxitin, ceftazidime, cefotaxime, cefotaxime, and cefotaxime/clavulanate, tigecycline (by MIC); trimethoprim/sulfamethoxazole, fosfomycin, nitrofurans, and other antibiotics from the beta-lactam class (namely carbapenemes), fluoroquinolones and aminoglycosides (by disc diffusion method). β-lactamase characterization was performed by isoelectric focusing, followed by PCR and sequencing of *bla* genes from Ambler class A (bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{\mathrm{CTX\text{-}M}}$, bla_{GES}, bla_{KPC}, bla_{SME}, bla_{NMC}), class C (bla_{MOX}, bla_{CIT}, bla_{DHA}, bla_{ACC}, bla_{MIR}, bla_{FOX}, bla_{ACT}), and class D ($bla_{\mathrm{OXA\text{-}48}}$). The *ram*R (*Klebsiella pneumoniae*) and *mar*R (*Escherichia coli*) efflux pump genes involved in tigecycline resistance were also investigated by PCR and sequencing. Positive controls were used in each method performed.

Results:

Susceptibility testing evaluation identified 73% of tigecycline susceptible isolates, and 89% of multidrug resistance, among which 70% were susceptible to this antibiotic. Although there was a high prevalence of β-lactam resistance, carbapenems were still effective against the majority of the isolates studied, as well as colistin and amikacin. Globally, molecular characterization allowed the detection of penicillinases, ESBLs from families CTX-M [CTX-M-15-(type), CTX-M-1, CTX-M-32 and CTX-M-14], TEM (TEM-4, TEM-10), SHV (SHV-2, SHV-12, SHV-55) and GES (GES-7), as well as carbapenemases (KPC-3) and AmpC β-lactamases (CMY-2, DHA-1, MIR-type); among those, 75% were susceptible to tigecycline. The molecular analyses of tigecycline resistance mechanisms revealed one deletion, one insertion and one to four point mutations in the *ramR* gene that might contribute to the overexpression of AcrAB efflux pump, in 9 out of 20 *Klebsiella pneumoniae* isolates showing reduced susceptibility. Considering the analyses of the *marR* gene from 12 *Escherichia coli* isolates (5 with and 6 without tigecycline resistance), two point mutations were detected.

Conclusions:

This study showed a great diversity of β-lactamases, such as ESBL, and the presence of carbapenemases in Gram negative bacteria. Nevertheless, the studied isolates still showed decisive susceptibility patterns to important antibiotic classes, namely tigecycline, carbapenems, colistin and amikacin. The modifications identified in genes regulating the AcrAB efflux pump are accountable for the tigecycline resistance, but also to several antimicrobial classes, contributing to the high multidrug resistance scenario.