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Paper Poster Session
The complexity of antibacterial resistance mechanisms

In vitro activity of tigecycline and resistance mechanisms due to efflux pump production in Gram-negative isolates in Portugal

Vera Manageiro*[1], Joana Almeida[2], Sofia Pires[2], Daniela Jones-Dias[2], Eugénia Ferreira[2], Manuela Caniça[2]

[1]*National Institute of Health, Department of Infectious Diseases, Lisbon, Portugal*

[2]*National Institute of Health Dr. Ricardo Jorge, Department of Infectious Diseases, Lisbon, Portugal*

Background: Tigecycline is increasingly used to treat infections caused by Gram negative isolates, because these bacteria express multidrug-resistance (MDR) phenotypes in an escalating frequency. Thus, this study aimed: 1) to correlate mediated □-lactam and fluoroquinolone resistance mechanisms (RMs) among Gram-negative isolates causing untreatable bacterial infections nowadays; 2) to study the *in vitro* activity of tigecycline to identify the importance of this antibiotic; 3) and to characterize tigecycline resistance mechanims (RM) in order to understand how to extend its activity.

Material/methods: A total of 2560 unduplicated Gram-negative (2309 *Enterobacteriaceae* and 251 *Acinetobacter baumannii*) isolates, collected in healthcare facilities in Portugal (2009-2013), were studied. MICs of tigecycline were determined against all isolates, and interpreted according to EUCAST (*Enterobacteriaceae*) or CLSI (*A. baumannii, Ab*) guidelines. Antimicrobial susceptibility of other 17 antibiotics was determined to all clinical isolates by disk diffusion method and interpreted by EUCAST. Fluoroquinolone and β-lactam RMs were reached by PCR and sequencing of 623 *Enterobacteriaceae* and 133 *Ab* using specific primers targeting PMQR-, Class A- and D β-lactamase-, Class B/MBL- and PMAβ-encoding genes. Tigecycline resistance due to efflux pump production was studied by molecular methods: *ramR* gene of *Klebsiella pneumoniae* and *marR* gene of *Escherichia coli* isolates.

Results: We identified 27.2%/35.8% and 96.4%/96.2% tigecycline non-susceptible/MDR *Enterobacteriaceae* and *Ab* isolates, respectively. The molecular analyses of tigecycline RMs revealed deletions/insertions/point mutations in the *ramR* gene that might contribute to the overexpression of AcrAB efflux pump in 63 out of 108 *K. pneumoniae* isolates showing reduced susceptibility to tigecycline. Point mutations observed in *marR* gene from *E. coli* isolates with or without tigecycline resistance, might contribute to MDR scenarios. A great diversity of β-lactamases was observed in *Enterobacteriaceae* isolates: penicillinases, ESBLs (CTX-M-1/-14/-15/-32/-G1-type/-G2-type, TEM-4/-10, SHV-12/-55, GES-7), carbapenemases (KPC-3, GES-5/-6, OXA-48, VIM-2/-34) and PMAβ (CMY-2, DHA-1, MIR-type, ACT-type); *Enterobacteriaceae* were fully susceptible to tigecycline for: 48.3% of β-lactamase producers; 65.6% carrying PMQR determinants; 69.1% presenting both RMs. Concerning *Ab* all 133 isolates tested (3.8% tigecycline susceptible) expressed an acquired carbapenemase (OXA-23 and/or OXA-24).

Conclusions: This study showed that tigecycline remains a substrate of MDR *Ab* isolates. However, the *in vitro* susceptibility of *Enterobacteriaceae* isolates to tigecycline showed its decisive importance when these bacteria presented resistance to other antibiotic classes, specifically through plasmid-mediated □-lactam and fluoroquinolone RMs. Thus, tigecycline contribution against the most important MDR Gram-negative pathogens should be preserved principally to bacterial infections untreatable by other antibiotics (such as carbapenems and colistin).