Contribution of a different pattern of stimulation of the innate immune system in virulence of *Klebsiella pneumoniae*-producing carbapenemase

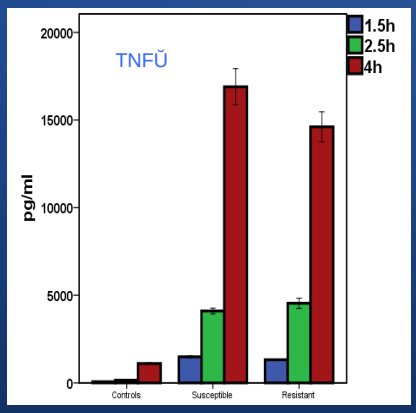
Iliana-Maria Pantelidou, Dionyssia-Pinelopi Carrer, Marianna Georgitsi, Evangelos J. Giamarellos-Bourboulis

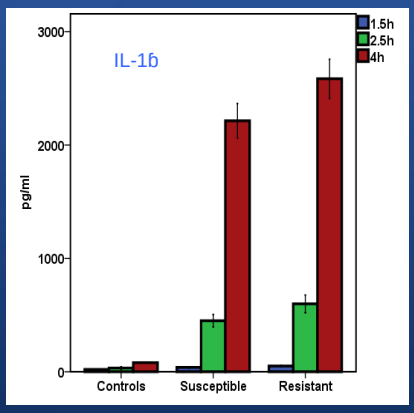
4th Department of Internal Medicine, University of Athens, Medical School, Greece

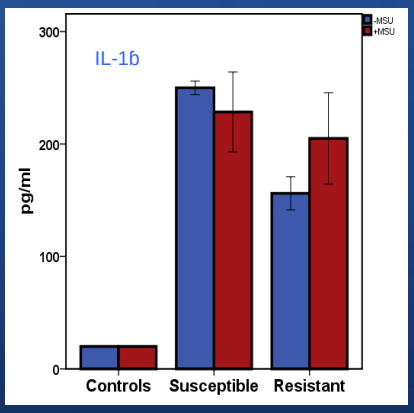
Purpose It is postulated that mortality of nosocomial infections by multidrug-resistant (MDR) *Klebsiella pneumoniae* is related to the lack of active antimicrobials. It was investigated if part of the effect of MDR may be related with the stimulation pattern of host immune responses.

Methods Twenty blood isolates were studied: 5 isolates susceptible to antimicrobials, 5 ESBL-producing, 5 VIM-producing and 5 KPC. Genetic diversity was defined by PFGE. Peripheral blood mononuclear cells of healthy volunteers were in vitro stimulated at a density of $5x10^6$ cfu/ml by live and heat-killed (HK) isolates for the production of pro-inflammatory cytokines in the presence of monosodium urate (MSU) that is a NLRP3-inflammasome agonist. Cytokines were measured by an enzyme immunoassay. Survival of 30 C57B6 male mice was recorded after intraperitoneal challenge with 3 susceptible and 3 KPC-producing isolates in 5 mice each.

Results Respective mean release of TNFa after 1.5 hours stimulation with live susceptible, ESBL-producing, VIM-producing and KPC isolates was 1492.1, 1144.3, 1459.5 and 1381.0 pg/ml; it became 4104.8, 4619.3, 4157.0 and 4791.8 pg/ml vs susceptible). Surprisingly, mean release of IL-1b after stimulation of after 2.5 hours. Respective mean release of IL-1b after 1.5 hours stimulation with live susceptible, ESBL-producing, VIM-producing and KPC isolates was 38.9, 35.3, 53.4 and 63.5 pg/ml; it became 499.9, 572.7, 517.8 and, 684.2 pg/ml after 2.5 hours (p: 0.017 KPC PBMCs with HK-susceptible and HK-KPC isolates was 383.2 and 311.9 pg/ml respectively; it was changed to 1902.8 and 2150.2 pg/ml after addition of MSU denoting significant synergy at the NLRP3-stimulation level. Median survival of mice challenged by the susceptible isolates was 166 hours as opposed to 122 hours after challenge by KPC isolates (log-rank: 4.908, p: 0.027).







Conclusions Considerable differences are encountered in the stimulation of human PBMCs by susceptible *K.pneumoniae* and KPC probably related with the potential for NLRP3 stimulation. These differences may have an impact on therapeutics.