Colistin-resistant Gram-negative pathogens lacking the mcr-1 gene isolated from clinical specimens of hospitalized patients in Turkey

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Introduction
During 1980s, due to their high nefrotoxicity, parenteral use of polymyxins was abandoned limiting their use only to topical and oral intake. As the MDR strains continue to be a grand problem, previously abandoned antibiotics has been reintroduced to clinics. However, in the last few years, several cases of colistin resistant strains were reported in many countries including Turkey. Recently a plasmid mediated colistin resistance gene (mcr-I) was described. Shortly after its discovery, existence of mcr-1 in different isolates were reported. Being mediated by a stable mobile genetic element, this newly discovered gene raises an alarming health concern. In Turkey, colistin has been commonly used for the treatment of the infections in hospitalized patients caused by only MDR gram-negative pathogens, especially in carbapenem resistant K. pneumoniae and MDR A. baumannii infections for about last five years.

Objective of the study
Regarding the epidemiological importance of colistin resistance, CoIR strains have been collected in our institution – four hospitals with 1300 bed capacity- since 2014. The objective of this study is to conduct the surveillance on CoIR Gram-negative pathogens isolated from different body fluids (e.g. blood, urine, endotracheal aspirate, abscess, wound and bile) of patients in a Turkish metropolitan hospital complex.

Method
Â ColIR Gram-negative pathogens were isolated from different clinical speciemens of patients (April 2014-March 2016).
Â The species identification and antimicrobial susceptibility tests of each isolate were conducted following conventional methods and Vitek-2 automatized system.
Â A colistin minimum inhibitory concentration is detected using Vitek-2 and confirmed by E-test.
Â For CoIR isolates, the presence of the recently discovered resistance gene was detected using PCR based on the primers described by Yi-Yun Liu et al.
Â The clonal relationship of the CoIR K. pneumoniae isolates were demonstrated based on the PFGE profiles of the strains. In addition, 3 CoIS-K. pneumoniae strains - isolated from the patients before CoIR pathogens isolation- were included in PFGE study.

Results
Â A total of 45 CoIR Gram-negative pathogens (37 K. pneumoniae, 5 A. baumannii, one A. junii, one P. aeruginosa and one C. brakii) were isolated from 45 different patients.
Â 29 (64.4%) of the patients were hospitalized in intensive care units.
Â All 45 isolates were found to be PCR-negative for mcr-1 gene.
Â According to PFGE, one major and four minor clones were found.
Â Two CoIS K. pneumoniae (Kp42 and Kp43) strains showed 100% PGFE similarities with one CoIR K. pneumoniae (Kp3) strain isolated from the same patients within 3 months.
Â Four out of 5 recently (2016) isolated CoIR strains (Kp36, Kp38, Kp39 and Kp40) do not belong to a previously defined clone showing up as STs. The similarity level between these recently isolated strains and the closest strains is about 75%.

Conclusions
Â Colistin is our last line of defense in the treatment of infections caused by MDR pathogens and up to now CoIRpathogens are only observed in clinics, in Turkey.
Â Based on PFGE, the presence of new singletons can be explained by the infiltration of new CoIR strains from outside into the clinic or by the molecular evolution of CoIS strains under colistin pressure.
Â Non-existence of mcr-I gene and PGFE profiles (same clone Col S/R isolates lacking mcr-I) might be an implication that the horizontal mechanism is not responsible for the resistant phenotype in our collection and the CoIR might be due to the other vertical mechanisms under colistin stress rather than a gene transfer across resistome-mobilome ecosystem.
Â However, other unknown CoIR genes mediated by mobile genetic elements might exist and be the actual reason of colistin resistance observed in modern pathogens.
Â Further genome-based studies are needed to discover unknown colistin resistance mechanisms to take measure in infection control regarding the last resort before the potential pan-resistance crisis.
Â As its dissemination raises a global risk for pan-drug resistant strains, it is crucial to conduct surveillance studies on the CoIR Gram-negative bacteria.