



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

Aycan Gundogdu<sup>1,2</sup>, Huseyin Kilic<sup>1</sup>, Aysegul Ulu-Kilic<sup>3</sup>

<sup>1</sup>Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey, <sup>2</sup> Genome and Stem Cell Center, Erciyes University, Kayseri, Turkey, <sup>3</sup> Department of Infectious Diseases and Clinical Microbiology,, Faculty of Medicine, Erciyes University, Kayseri



## Introduction

During 1980s, due to their high nephrotoxicity, 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-1*) 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, ColR 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 ColR 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

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

## Results

- A total of 45 ColR Gram-negative pathogens (37 *K. pneumoniae*, 5 *A. baumannii*, one *A. junii*, one *P. aeruginosa* and one *C. braakii*) were isolated from 45 different patients.
- 29 (64.4%) of the patients were hospitalized in intensive care units.
- All 45 isolates were found to be PCR-negative for *mcr-1* gene.
- According to PFGE, one major and four minor clones were found.
- Two ColS *K. pneumoniae* (Kp42 and Kp43) strains showed 100% PGFE similarities with one ColR *K. pneumoniae* (Kp3) strain isolated from the same patients within 3 months.
- Four out of 5 recently (2016) isolated ColR 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%.

## References

Ah YM, Kim AJ, Lee JY. Colistin resistance in Klebsiella pneumoniae. Int J Antimicrob Agents. 2014;44(1):8-15. Keske S, Yilmaz GR, Güven T et al. A health care-associated pneumonia case due to colistin resistant Acinetobacter baumannii. J Microbiol Infect Dis. 2014;4(3):111-113. Liu Y-Y, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2015;3099:1-8. Arcilla MS, van Hattem JM, Matamoros S et al and COMBAT consortium. Dissemination of the mcr-1 colistin resistance gene. Lancet Infect Dis 2015. http://dx.doi.org/10.1016/S1473-3099(15)00541-1. Adams MD, Nickel GC, Bajaksouzian S, et al. Resistance to colistin in Acinetobacter baumannii associated with mutations in the PmrAB two-component system. Antimicrob Agents Chemother. 2009;53:3628-34.



Characteristics of colistin resistant micro.

Pathogens	No
<i>K. pneumoniae</i> *	37
<i>A. baumannii</i>	5
<i>A. junii</i>	1
<i>C. braakii</i>	1
<i>P. aeruginosa</i>	1
Clinical Samples	
Blood & Catheter	13 & 2
Urine	9
Wound & Abscess	5 & 1
ETA**	5
Tissue	3
Bile	2
Nephrostomy	2
BAL***	1
Sputum	1
colistin use	
none	18
1-7 days	8
7-14 days	6
14-21 days	6
>21	9

\*pathogens have carbapenamase and/or ESBLs activities, \*\* ETA; endotracheal aspirate, \*\*\*BAL; bronchoalveolar lavage.

## Conclusions

- Colistin is our last line of defense in the treatment of infections caused by MDR pathogens and up to now ColR pathogens are only observed in clinics, in Turkey.
- Based on PFGE, the presence of new singletons can be explained by the infiltration of new ColR strains from outside into the clinic or by the molecular evolution of ColS strains under colistin pressure.
- Non-existence of *mcr-1* gene and PGFE profiles (same clone Col S/R isolates lacking *mcr-1*) might be an implication that the horizontal mechanism is not responsible for the resistant phenotype in our collection and the ColR might be due to the other vertical mechanisms under colistin stress rather than a gene transfer across resistome-mobilome ecosystem.
- However, other unknown ColR genes mediated by mobile genetic elements might exist and be the actual reason of colistin resistance observed in modern pathogens.
- 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.
- As its dissemination raises a global risk for pan-drug resistant strains, it is crucial to conduct surveillance studies on the ColR Gram-negative bacteria.