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Clonal relationship between NDM-1-positive *Klebsiella pneumoniae* isolates collected from 14 Turkish hospitals

Onur KARATUNA[1, 2], Deniz Ece KAYA[1], Sesin KOCAGÖZ[3], Meltem KAYA[2], Işın AKYAR[1, 2], Tanıl KOCAGÖZ[1]

[1] Acıbadem University School of Medicine, Department of Medical Microbiology, Istanbul, Turkey

[2] Acıbadem Labmed Medical Laboratories, Istanbul, Turkey

[3] Acıbadem University School of Medicine, Department of Infectious Diseases and Acıbadem Maslak Hospital, Istanbul, Turkey

Objectives

New Delhi metallo-beta-lactamase-1 (NDM-1) carbapenemase-producing *Enterobacteriaceae* isolates are considered as a major health threat. NDM-1-producing *Klebsiella pneumoniae* isolates are being reported from Turkish hospitals but the epidemiological characterization of these isolates is lacking. This study investigates the clonal relationship of *bla*$_{NDM-1}$ positive isolates among carbapenem-resistant *K. pneumoniae* collected from four major Turkish cities (Istanbul, Adana, Eskisehir, Kocaeli) between January 2012-May 2015, including the first reported NDM-1 *K. pneumoniae* isolate in Turkey.

Methods

The initial susceptibility testing was performed with VITEK 2 automated antimicrobial susceptibility test system (bioMérieux, France). For isolates which exhibit resistance to any of the tested carbapenems (i.e. ertapenem, imipenem, meropenem), the carbapenemase genes (IMP, VIM, NDM-1, KPC, and OXA-48) were investigated with an in-house polymerase chain reaction (PCR) test. For the investigation of clonal relationship between NDM-1-positive *K. pneumoniae* isolates (n=64) which were collected from 13 different hospitals plus the first reported NDM-1-positive *K. pneumoniae* isolate in Turkey detected at a hospital in Kocaeli in October 2011, pulsed-field gel electrophoresis (PFGE) was used: DNA was extracted from bacteria grown in tryptic soy broth and agar disks were prepared by embedding DNA in low melting agarose.

Disks were incubated overnight with proteinase K at 50°C. Digested genomic DNA inside the disks with Xba-1 was loaded into 1% agarose gel in 0.5x TBE solution and was run on CHEF-DR II instrument (Bio-Rad Laboratories, USA) with the following parameters: initial time 5 sec, final time 35 sec, 6.0 V/cm for 23 h and visualised with ethidium bromide in ChemiDoc XRS system (Bio-Rad Laboratories, USA).

Results

PFGE of Xba-1-digested genomic DNA of *bla*$_{NDM-1}$ positive isolates revealed 17 distinct pulsotypes among *K. pneumoniae* isolates, 55 isolates were distributed into seven pulsotypes and the remaining 10 isolates, including the first reported NDM-1-positive *K. pneumoniae* isolate, exhibited unrelated pulsotypes (Table 1).

The pulsotype 1 included 21 of the isolates, 20 being from the same hospital.

Conclusion

Our results suggest that the first NDM-1-positive *K. pneumoniae* isolate did not spread to other hospitals investigated in this study.

Although the pulsotype 1 contains the biggest number of isolates, it seems to be contained within the hospital B.

The pulsotype 6 clone exhibited the widest distribution, it was detected in four hospitals, three from Istanbul, one from Eskisehir.

When hospitals in the European side of Istanbul are compared to Asian side hospitals, it is clear that in the European side of the city, NDM-1-positive *K. pneumoniae* is spreading more easily and quickly, both between patients and institutions.

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Table 1. Clonal relationship between NDM-1-positive *Klebsiella pneumoniae* isolates (n=65) collected from 14 Turkish hospitals

Hospital Code	City	No. isolates	First - Last Isolation Date	PFGE Pulsotype							
				1	2	3	4	5	6	7	Unrelated
A	Kocaeli	1	Oct 2011								1
B	Istanbul (Europe)	31	Aug 2014 - May 2015	20	7			2	2		
C	Istanbul (Europe)	7	May 2014 - Dec 2014				3	2		2	
D	Istanbul (Europe)	5	Aug 2012 - May 2015				3				2
E	Istanbul (Europe)	4	Jan 2012 - Aug 2012			4					
F	Istanbul (Europe)	4	Aug 2012 - Nov 2013			1			2		1
G	Istanbul (Europe)	4	Jul 2012 - Apr 2015					1	2		1
H	Eskisehir	2	December 2014						1		1
I	Kocaeli	2	Feb 2014 - Apr 2015							1	1
J	Istanbul (Europe)	1	Sep 2014								1
K	Adana	1	May 2013								1
L	Istanbul (Asia)	1	Dec 2013			1					
M	Istanbul (Asia)	1	Jan 2014								1
N	Istanbul (Asia)	1	Apr 2015	1							

PFGE: Pulsed-field gel electrophoresis