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Oral Session
Molecular detection of resistence: dream or reality?

THE TALE OF TWO OUTBREAKS: MONO- VS. MULTI-SPECIES COLONIZATION BY KPC-PRODUCING ENTEROBACTERIACEAE

E. Khabra[1], A. Adler[1], S. Paikin[2], Y. Carmeli[1]

[1]Epidemiology, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel ; [2]Microbiology, Laniado Hospital, Netanya, Israel

Objectives: The carbapenemase KPC has disseminated globally mainly by clonal spread of the epidemic KPC-producing *Klebsiella pneumoniae* (KPC-KP) ST-258 clone. The *bla*$_{KPC}$ gene may also spread by horizontal gene transfer (HGT), as suspected when more than one KPC-producing Enterobacteriaceae species (KPC-Ent) is isolated in a single patient at the same time. In this study, we aimed to examine the incidence and characterize the molecular features of KPC-Ent, in cases where multiple KPC-Ent are detected from the same patient, in comparison with carriage of isolated KPC-KP.

Methods: The incidence of KPC-Ent cases was recorded from April 2011 until August 2012 at the Laniado Hospital (LH). The *bla*$_{KPC}$ gene allele was determined by sequencing. Full molecular characterization was done in all cases of multiple KPC-Ent and on a random sample of KPC-KP isolated alone (n=14). Typing was done by PFGE and MLST. Plasmids were characterized by replicon typing, plasmid MLST (pMLST), S1-nuclease analysis and RFLP. The genetic environment of the *bla*$_{KPC}$ gene was studied by PCR for the flanking regions of the *Tn*4401 transposon and sequencing.
Results: The molecular characteristics of KPC-producing *K. pneumoniae*, isolated alone or with other KPC-Ent are presented in the table. During the 17-month period, the incidence of isolated KPC-KP was 274 (16.1/month), of which 31 were from clinical culture and 243 from rectal surveillance cultures. Eight patients had multiple KPC-Ent (table), of which seven were detected in surveillance culture and one in urine culture. All KPC-KP isolated alone (n=14) belonged to the epidemic ST-258 clone and carried the *bla*$_{KPC-3}$ allele that was harbored mainly by IncFII-type plasmids. On the other hand, KPC-KP's that were isolated together with other KPC-Ent belonged to various ST's and carried the *bla*$_{KPC-2}$ allele (in 7/8 isolates). The *bla*$_{KPC-2}$ gene was located inside a 5-kb transposon flanked by unique insertion sequences, harbored by IncN, pMLST ST-15 type plasmids of variable sizes. These plasmids were identical in 2 paired KPC-Ent cases.

Conclusions: This study highlights that two molecularly distinct outbreaks of KPC-Ent were occurring simultaneously. The higher scale outbreak was characterized by the spread of the *bla*$_{KPC-3}$ -producing, ST-258 epidemic *K. pneumoniae* clone, while the less common outbreak was characterized by HGT-related, multispecies dissemination of the *bla*$_{KPC-2}$ gene via various IncN, pMLST ST-15 type plasmids.

Table. Molecular characteristics of KPC-producing *K. pneumoniae*, isolated alone or with other KPC-producing *Enterobacteriaceae*

	KPC-KP isolated alone[1]	**KPC-KP isolated with additional KPC-Ent**
incidence	274	8
Additional KPC-Ent	None	E. coli (6), Enterobacter spp. (2), *Citrobacter freundii* (n=1)
bla$_{KPC}$ allele	*bla*$_{KPC-3}$	*bla*$_{KPC-2}$ (7), *bla*$_{KPC-3}$ (1)
KPC-KP clone	ST-258	Variable, non-ST-258
bla$_{KPC}$-containing transposon size	10 kb (*Tn*4401)	5 kb
bla$_{KPC}$-harboring plasmid type	Mainly IncFII-type	IncN, pMLST ST-15, variable size

[1]-molecular characteristics were studied in 14 isolates.