02302

Emergence of KPC variants from clinical origin associated with reduced susceptibility to ceftazidime- avibactam, including four novel blaKPC-2 variants

03. Bacterial susceptibility & resistance

3d. Resistance mechanisms incl in vitro and in vivo studies, mobile elements (excl TB)

Fernando Pasteran [1], Sonia Gomez [1], Belen Sanz [2], Juan Manuel De Mendieta [1], Diego Faccone [1], Laura Errecalde [3], Maria Rosa Nuñez [4], Renata Monge [5], Valeria Alexander [6], Maria Daniela Cudmani [7], Magdalena Pennini [8], Alejandra Corso [1]

[1]Antimicrobianos INEI ANLIS Malbrán - Caba (Argentina), [2]Antimicrobianos INEI ANLIS Malbrán - Caba (Argentina) - Caba (Argentina), [3]Hospital Juan A Fernández - Caba (Argentina), [4]Hospital Castro Rendón - Neuquén (Argentina), [5]Hospital Británico de Bs As - Caba (Argentina), [6]Hospital Italiano de Bs As - Caba (Argentina), [7]Laboratorio Central de Salud Pública - Tucuman (Argentina), [8]Laboratorio Stamboulian - Caba (Argentina)

Background KPC-producing Enterobacterales are a great health concern and therapy with ceftazidime-avibactam (CZA) represents, by the moment, one of the best choices for the treatment of infections supported by these strains. We report the emergence of CZA resistance in six Klebsiella spp. KPC producers. We also report on the identification of four novel blaKPC variants, named blaKPC-80, blaKPC-81, blaKPC-96 and blaKPC-97.

Methods Six clinical isolates were referred to NRL due to resistance to CZA in routine testing, among metallo-lactamase non-producers (confirmed by the lack of EDTA inhibition). Specie identification was confirmed by MALDI-TOF MS. MICs were determined by agar dilution (CLSI). blaKPC, blaOXA-48-like, blaNDM, blaVIM and blaIMP were screened by an NRL-developed multiplex PCR. WGS was carried out using Illumina. KPC variants were further confirmed by Sanger sequencing. Carbapenemase production was investigated by: Triton Hodge test, Blue Carba, Carba NP, modified Carbapenem Inactivation, BD CPO Detect and immunochromatographic assays.

Results CZA resistance was confirmed in all six Klebsiella spp. isolates (MICs range: 16 - >256 mg/L). Only one patient was previously treated with CZA. Isolates carried blaKPC-2 alleles with either: N240Y mutation (blaKPC-96), D273-SEAVN-277 deletion (blaKPC-97), 45 nucleotide insertions after position 259 (blaKPC-44), D179V mutation (blaKPC-57), D266-PNK-268 deletion (blaKPC-80) or D172-I deletion (blaKPC-81). All except one isolate belonged to the CC11 hyper-epidemic clonal complex. Colistin resistance was due to pmrB_R256G mutation. Only two

strains were fully susceptible to carbapenem, but all isolates regained carbapenem or aztreonam susceptibility in the presence of avibactam or relebactam. Fosfomycin was the most active non-beta-lactam antibiotic. None of the carbapenemase detection assays allowed to unequivocally identify the isolates as carbapenemase producers. Immunochromatographic assay required up to 60 minutes to reveal a faint KPC band in the 3 strains identified as positive. Detail results in Figure 1.

Conclusions These findings raise relevant issues: 1) novel KPC-type enzymes conferring resistance to CZA were identified, including four new allelic variants; 2) emergence of KPC variants may represent a challenge for antimicrobial management; 3) more common carbapenemase detection methods were unable to detect KPC. Thus, we advocate routine CZA susceptibility testing of Enterobacterales, even in the absence of prior drug exposure.

Strain	**25197**	**25752**	**25399**	**25802**	**25775**	**25923**
KPC type	***KPC-96***	***KPC-81***	***KPC-80***	***KPC-44***	***KPC-57***	***KPC-97***
Hospital	Juan A Fernandez	Castro Rendon	Britanico	Italiano Bs As	Lab Central Salud Publica	Stambulian
City	CABA	Neuquen	CABA	CABA	Tucuman	CABA
Date of isolation (m-d-y)	10/10/19	11/12/19	7/10/20	7/7/20	8/28/20	12/11/20
Bacterial specie	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. pneumoniae*	*K. aerogenes*	*K. pneumoniae*
MLST-type	258	258	629	11	-	11
Site of isolation	urine	abdominal	urine	rectal (screening)	BAL	rectal (screening)
Antimicrobial susceptibility (MICs in mg/L)						
Imipenem	4	0,5	4	32	1	4
Imipenem + avibactam	0,008	0,12	0,12	0,5	1	0,5
Imipenem + relebactam	0,12	0,12	0,25	1	0,5	1
Meropenem	0,5	0,25	0,5	12	0,25	2
Meropenem + avibactam	0,008	0,12	0,032	1	0,25	0,125
Ceftazidime	32	>256	128	>256	>256	>256
Ceftazidime + avibactam	16	>256	128	64	>256	>256
Ceftazidime + clavulanic acid	64	16	32	>256	8	256
Cefotaxime	8	4	16	64	8	16
Cefotaxime + avibactam	0,5	0,5	0,5	4	4	2
Cefotaxime + clavulanic acid	8	0,12	4	32	4	8
Ceftaroline	32	32	16	32	16	128
Ceftaroline + avibactam	0,25	2	4	4	8	8
Aztreonam	128	16	32	48	4	64
Aztreonam + avibactan	0,125	0,5	0,25	0,5	2	1
Ceftolozane + tazobactam	>256	>256	>256	>256	>256	>256
Other antibiotics:						
Susceptible	AMK, FOS	FOS, FOX	AMK, FOS, COL, FOX	AMK, FOS, COL	AMK, COL	FOS, FOX
Resistant	COL, GEN, CIP, FOX, SXT, TIG	AMK, COL, GEN, CIP, SXT, TIG	GEN, CIP, SXT, TIG	GEN, CIP, FOX, SXT, TIG	FOS, GEN, CIP, FOX, SXT, TIG	AMK, COL, GEN, CIP, SXT, TIG
Carbapenemase production test						
K.O.O Lateral flow	NEG	NEG	KPC	KPC	NEG	KPC
Blue Carba, Carba NP	NEG	NEG	NEG	NEG	NEG	NEG
mCIM	POS	NEG	POS	NEG	NEG	NEG
THT	POS	NEG	POS	NEG	POS	POS
CPO test	Class A	Class A + ESBL	Class A	Carbapenemase	Class B	Class A

Abbreviations: AMK: amikacin, FOS: fosfomycin, COL: colistin, GEN: gantamicin, CIP: ciprofloxacin, FOX: cefoxitin, SXT, TIG: tigecycline. NEG: negative. POS: positive, MLST: multi-locus sequence type. K.O.O: immunochromatographic assay, mCIM: modified Carbapenem Inactivation, THT: Triton Hodge test.