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Emergence of carbapenemase-producing *Klebsiella pneumoniae* clinical isolates with a pan-drug resistant (PDR) phenotype in Argentina

03. Bacterial susceptibility & resistance

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

Likely attendance

Onsite

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Background

Carbapenemase producers represented the 33% of *K. pneumoniae* (Kpn) infections in Argentina 2021, being bla_{NDM} and bla_{KPC} responsible of 88% of those infections. NDM producers are usually susceptible to aztreonam plus ceftazidime/avibactam (AZA). We describe here the emergence of three closely related Kpn clinical isolates with a PDR phenotype, including high-level resistance to AZA.

Methods

For antimicrobial susceptibility testing we used: 1) agar dilution for AZA, ceftazidime/avibactam (CZA), imipenem/relebactam (IMR) (DBOs 4mg/L) and rifampin; 2) cefiderocol microdilution with iron depleted CAMHB; 3) colistin disks elution and 4) susceptibility to other antimicrobials, through automated systems. Synergistic activity of selected antimicrobials was assessed crossing gradient diffusion strips at a 90° angle at the intersection of each MIC. Synergy tests were carried-out on MHA plates, alone or supplemented with the indicated drug/DBOs. Relevant genes were characterized by two multiplex PCR (*bla*KPC/*bla*NDM/*bla*OXA-48-like/*bla*VIM/*bla*IMP and *bla*CTXM/*bla*PER/*bla*CIT). NGS was performed by Illumina MiSeq. MLST and alleles were confirmed by assemblies in silico and AMRFinder/ARIBA and/or by Sanger sequencing.

Results

Kpn1 to Kpn3 were recovered in a single Institution from urine samples of three respective patients undergoing kidney transplantation (male, 38-54 y.o). Strains were only susceptible to cefiderocol (Fig-1), which is not available in Argentina. Patients remained alive after compassionately treatment with a triple combination of aztreonam plus CZA plus clavulanic

acid (Fig-2) but the transplanted kidneys were rejected. Strains were confirmed as ST258, capsular type K107/O1/O2V2/wzi154. SNP analysis revealed a close evolutionary relationship between isolates: 59 SNP (Kpn1 vs Kpn2), 47 SNP (Kpn2 vs Kpn3) and 90 SNP (Kpn1 vs Kpn3). The 3 strains were confirmed as producers of $bla_{\text{NDM-5}}$, $bla_{\text{CTXM-15}}$, $bla_{\text{SHV-5-like}}$ (K234R, A237G), additionally, Kpn2 co-produced $bla_{\text{KPC-2}}$ and $bla_{\text{OXA-1}}$. Main resistance mechanisms are depicted in Fig-3.

Conclusions

We described the emergence of Kpn isolates resistant to all antibiotics available in the country. PDR was multicausal, involving β-lactamases, modifying enzymes, efflux, porin deficiency and target mutations, including essential PBPs. The addition of two β-lactamase inhibitors was required to reduce aztreonam MICs to levels close to the PK/PD breakpoint for continuous infusion. The emergence of PDR strains should be considered of high epidemiological risk, maximizing efforts for rapid detection and containment.

Fig-1. Antimicrobial susceptibility profile

	Kpn1 Date: 08-28-2022		Kpn 2 Date: 09-01-2022		Kpn3 Date: 09-15-2022	
	MIC	Cat.	MIC	Cat.	MIC	Cat.
Aminopenillins, Piperacillin+tazobactam extended spectrum cephalosporins, cefoxitin	>64	R[1]	>64	R[1]	>64	R[1]
Meropenem, imipenem, ertapenem	>32	R[1]	>32	R[1]	>32	R[1]
Amikacin, gentamicin	>32	R[1]	>32	R[1]	>32	R[1]
Ciprofloxacin, levofloxacin	>4	R[1]	>4	R[1]	>4	R[1]
Minocycline	>8	R[1]	>8	R[1]	>8	R[1]
Tigecycline	>2	R[2]	>2	R[2]	>2	R[2]
Fosfomycin	>64	R[2]	>64	R[2]	>64	R[2]
Trimethoprim sulfamethoxazole	>2	R[1]	>2	R[1]	>2	R[1]
Rifampin	>4	R[3]	>4	R[3]	>4	R[3]
Nitrofurantoin	>64	R[1]	>64	R[1]	>64	R[1]
Chloramphenicol	>16	R[1]	>16	R[1]	>16	R[1]
CZA, IMR	>32	R[1]	>32	R[1]	>32	R[1]
Colistin	>8	R[2]	>8	R[2]	>8	R[2]
Aztreonam	>256	R[1,2]	>256	R[1,2]	>256	R[1,2]
AZA	32	R[4]	>256	R[4]	>256	R[4]
Cefiderocol[5]	0,25	**S**[1,2]	0,25	**S**[1,2]	0,25	**S**[1,2]

S: susceptible. R: resistant
1. CLSI breakpoint (M100, Ed 32-2022). 2 EUCAST breakpoint (v12, 2022)
3. Société Française de Microbiologie. 4. EUCAST breakpoint for aztreonam alone.
5. Cefiderocol is not available in Argentina

Fig.2 Evaluation of therapeutic options

SELECTED ANTIMICROBIAL/INHIBITOR COMBINATIONS				MICs values in mg/L.		
COMPOUND 1	COMPOUND 2	COMPOUND 3	COMPOUND 4	Kpn1	Kpn2	Kpn3
AZTREONAM	AVIBACTAM 4ug/ml	-	-	32	>256	>256
AZTREONAM	AVIBACTAM 4ug/ml	CLAVULANIC Ac. 4ug/ml	-	**1**	**24**	**32**
AZTREONAM	AVIBACTAM 10ug/ml	CLAVULANIC Ac. 4ug/ml	-	**1**	**24**	**32**
AZTREONAM	AVIBACTAM 4ug/ml	COLISTIN 1 ug/ml	-	24	>256	>256
AZTREONAM	AVIBACTAM 4ug/ml	FOSFOMYCIN 10ug/ml	-	24	>256	>256
AZTREONAM	AVIBACTAM 4ug/ml	RIFAMPIN 4ug/ml	-	32	>256	>256
AZTREONAM	RELEBACTAM 4ug/ml	-	-	24	>256	>256
AZTREONAM	RELEBACTAM 4ug/ml	CLAVULANIC Ac. 4ug/ml	-	**1**	**12**	**12**
AZTREONAM	AVIBACTAM 4ug/m	RELEBACTAM 4ug/ml	-	24	>256	>256
AZTREONAM	AVIBACTAM 4ug/m	RELEBACTAM 4ug/ml	CLAVULANIC Ac. 4ug/ml	**0,047**	**12**	**12**
MEROPENEM	COLISTIN 1ug/ml	-	-	>32	>32	>32
MEROPENEM	RIFAMPIN	-	-	>32	>32	-
AZTREONAM	AVIBACTAM 4ug/m	CLAVULANIC Ac. 4ug/ml	COLISTIN	-	**8**	**8**
AZTREONAM	AVIBACTAM 4ug/m	CLAVULANIC Ac. 4ug/ml	TIGECYCLINE	-	**16**	**16**
Most active combinations are highlighted in green						

Fig-3. Main resistant mechanisms identified by NSG

		Presence/absence of resistance genes and/or chromosomal mutations																													
		AMINOGLYCOSIDES				β-LACTAMICS							TETRACYCLINES		PHENICOL	TRIMETHOPRIM	SULFONAMIDES	RIFAMICINAS	QUINOLONES		MACROLIDES		QUATERNARY AMMONIUM	FOSFOMYCIN	EFFLUX PUMPS		COLISTIN		PBP3 (*ftsI*)		PBP2 (*mrdA*)	
	ST	*aac(6')-Ib*	*aac(3)-IId*	*aadA2*	*rmtB1*	bla_{TEM-1}	$bla_{SHV-5-like}$	bla_{OXA-1}	$bla_{CTX-M-15}$	bla_{KPC-2}	bla_{NDM-5}	*ompK36_D135DGD*	*rpsJ_V57L*	*tet(D)*	*catB3*	*dfrA12*	*sul1*	*arr-3*	*gyrA_S83I*	*parC_S80I*	*mph(A)*	*erm(B)*	*qacEΔ1*	*fosA*	*oqxA*	*oqxB*	*pmrB_R256G*	*pmrB_T140P*	*V375A*	*A413V*	*D354A*	*T529N*
Kpn1	258																														
Kpn2	258																														
Kpn3	258																														
		Presence of the indicated mechanism is highlighted in red																													

Keyword 1
Antibiotic stewardship (AMS)
Keyword 2
Antimicrobial resistance (AMR)
Keyword 3
pan-drug resistance

Conflicts of interest

Do you have any conflicts of interest to declare?
No