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3. Bacterial susceptibility & resistance Poster Session
3b. Resistance surveillance & epidemiology: Gram-negatives

Prevalence of mutations within RamAR operon associated with cross resistance phenotype among ESBL *Enterobacteriaceae* isolated in intensive care units

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BACKGROUND:

Enterobacteriaceae producing ESBL (ESBL-E) are a major public health problem leading to an increase of morbi-mortality. *Enterobacteriaceae* can adapt to their hosts and/or hospital environment through diverse mechanisms, in particular modification of efflux systems expression conferring resistance to a large panel of biocides.

AcrAB-TolC is a resistance-nodulation-division (RND) pump, which is in part regulated by a two-component system: RamAR operon. Mutations in this operon drive to a pump over-expression leading to a cross-resistance (CR) phenotype regarding quinolones, tigecycline, chloramphenicol, temocillin as well as a higher tolerance to biliary salts and resistance to phagocytosis.

The aim of the study was to determine the prevalence of the mutations inside the RamAR operon leading to a CR phenotype among ESBL-E isolated and sequenced in a French Teaching hospital.

MATERIAL & METHODS:

Throughout 2019, we studied the proportion of CR phenotype associated with a RamAR mutation among ESBL-E isolated from ICUs patients in a French University hospital^(1, 2).

- ✓ All the first ESBL-E isolated among patients hospitalized in medical, surgical or pediatric ICUs during 2019 have been collected and their genome sequenced by Illumina technology.
- ✓ Cross-resistances to ciprofloxacin, tigecycline, temocillin and chloramphenicol were searched by broth microdilution using EUCAST breakpoints.
- ✓ For each strain which presented a CR phenotype to the four molecules, MICs have been determined in presence of 20mg/L PAβN to highlight involvement of a RND pump in the CR phenotype observed.
- ✓ At the same time RamAR operon mutations have been looked for all the sequenced strains.
- ✓ Finally phenotypical and genomical data have been put together in order to find all the relevant RamAR mutation regarding the CR phenotype.

RESULTS:

In total 165 BLSE-E have been included. Thirteen strains presented a CR phenotype of which (7/49) *Enterobacter cloacae* complex, (6/53) *Klebsiella pneumoniae*, whereas none of the 63 *E.coli* tested presented the expected phenotype. MICs slightly decreased with addition of PAβN suggesting an RND pumps deregulation (TABLE 1). For all strains, no resistance gene could explain the CMIs increased of CR phenotype, but nine of them presented mutation in *ramR* gene. Mutations such as nucleotides substitutions or deletions were **specific to resistant strains** and affected the helix-turn-helix site of the protein, which drive to a disability of RamR to bind and inhibit the *ramA* expression.

TABLE 1: Characteristics of the 13 *Enterobacteriaceae* ESBL with cross-resistance to CIP, CHL, TEM, TIG

Species	Strain	cgMLST	MIC (mg/L)				Resistome				RamR modification			
			Alone				+ PAβN (20mg/L)							
			CIP	CHL	TEM	TIG	CIP	CHL	TEM	TIG	β-lactams	Quinolones	CHL	
<i>Enterobacter cloacae</i>	ECC_2019_0609	ST66	16	32	16	4	8	4	16	1	CTX-M-15	aac(6)-1b-cr, qnrB1	/	p.V1W fs D81**
	ECC_2019_1003	ST66	16	32	16	4	8	4	32	1	CTX-M-15	aac(6)-1b-cr, qnrB1	/	p.V1W fs D81**
	ECC_2019_0808	ST66	16	32	16	2	8	4	64	1	CTX-M-15	aac(6)-1b-cr, qnrB1	/	-
	ECC_2019_0903	ST66	16	32	16	2	8	4	32	1	CTX-M-15	aac(6)-1b-cr, qnrB1	/	-
	ECC_2019_0904	ST66	8	32	16	2	4	4	32	1	CTX-M-15	aac(6)-1b-cr, qnrB1	/	-
	ECC_2019_0501	ST106	4	32	32	4	2	4	32	2	SHV-12	/	/	p.29_32del
	ECC_2019_0702	ST114	256	>256	32	4	64	128	16	1	CTX-M-15	aac(6)-1b-cr, qnrB1, gyrA-S83L	/	p.1_69del
<i>Klebsiella pneumoniae</i>	KP_2019_0602	ST15	256	64	32	4	128	4	32	2	CTX-M-15	aac(6)-1b-cr, oqxAB	/	p.L159Q
	KP_2019_0706	ST15	64	16	32	2	32	< 2	32	0.5	CTX-M-15	aac(6)-1b-cr, oqxAB	/	p.L159Q
	KP_2019_1103	ST45	16	32	16	2	8	8	32	1	CTX-M-15	aac(6)-1b-cr, oqxAB, qnrB1	/	-
	KP_2019_0626	ST307	128	64	16	4	128	8	16	1	CTX-M-15	aac(6)-1b-cr, oqxAB, qnrB1	/	p.Y52N
	KP_2019_0803	ST405	32	128	16	2	8	16	32	0.5	CTX-M-15	aac(6)-1b-cr, oqxAB, qnrB1	/	p.W94R
	KP_2019_0403	ST449	0.5	128	32	4	< 0.125	8	32	1	SHV-2	oqxAB	/	p.1_64del

CIP: ciprofloxacin, CHL: chloramphenicol, TEM: temocillin, TIG: tigecycline

** Footnote: shift of the DNA reading frame by T567 deletion leading to protein RamR(L_8 VARPKSED>WHVRRVKI)

CONCLUSION:

In 2019 in our ICUs wards, approximately 10% of the ESBL-expressing *E.cloacae* and *K.pneumoniae*, showed mutations in their *ramR* regulator, disabling this gene. This suggests that RamR could be an important vector of antimicrobial resistance.

References:

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