

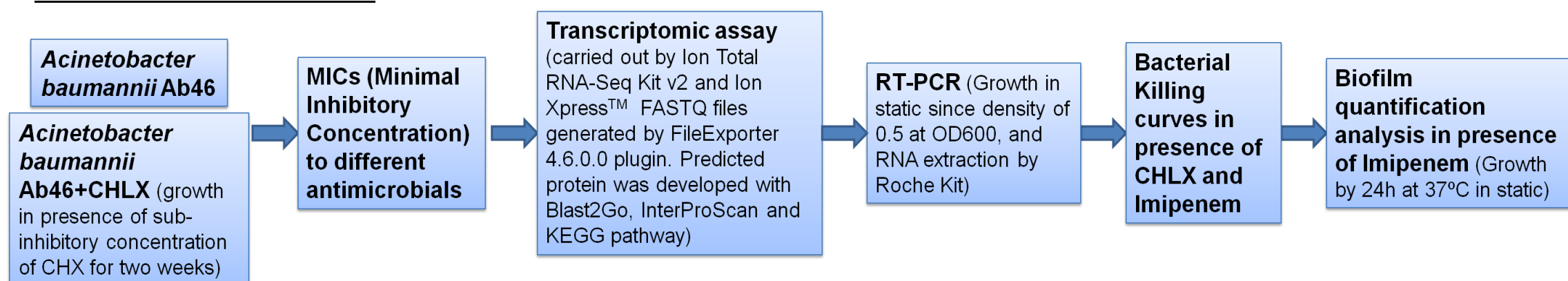
Expression of mechanisms of bacterial tolerance under chlorhexidine pressure in *A.baumannii* ST-2 clinical strain

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Introduction: Mechanisms of bacterial persistence and/or tolerance have been described under environmental stress and antimicrobial exposure. Chlorhexidine (CHLX) is a broad-spectrum antimicrobial agent with wide application. *A.baumannii* isolates from some health care settings can survive CHLX concentrations of at least 1%. However, very little is known of these mechanisms of persistence to CHLX in *A.baumannii* isolates. Using transcriptomic and microbiological studies, we analyzed these bacterial persistence/tolerance mechanisms in presence of CHLX in Ab46 *A.baumannii* strain from ST-2 clinical clone which harbor the toxin-antitoxin system (AbKA/AbkB) plasmidic.

Material and methods:



Results:

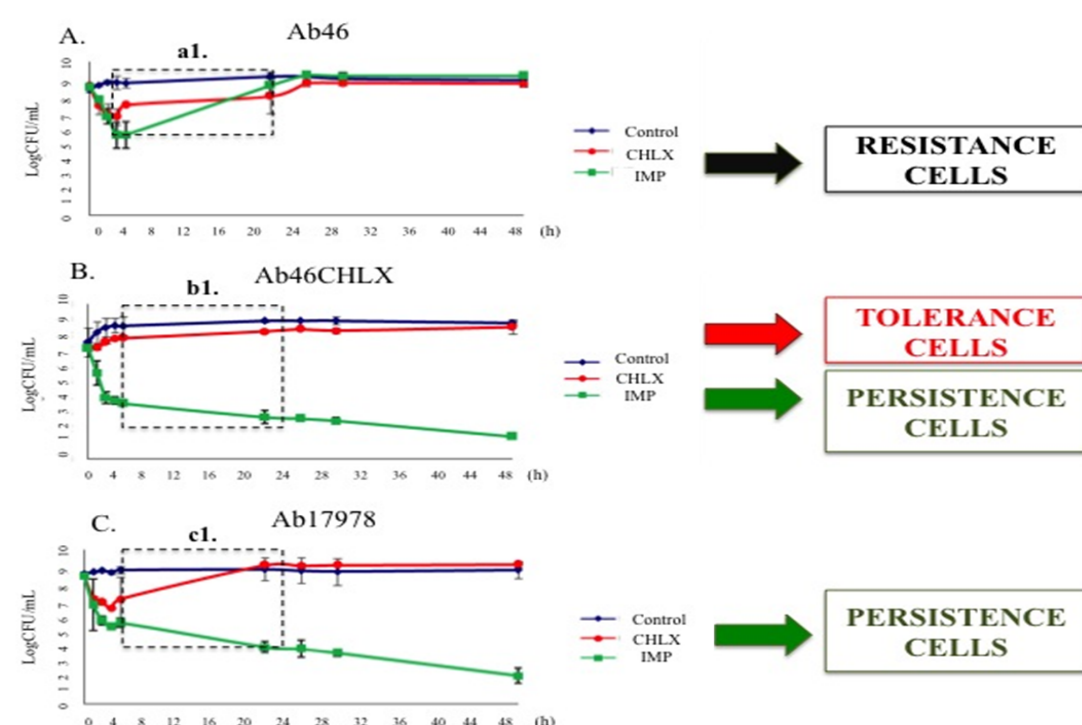
-In the Table 1a. MICs to several antimicrobials of both strains (Ab46 and Ab46+CHLX). In the Table 1b. MICs to CHLX of Ab46 before and after exposed to biocide.

a)

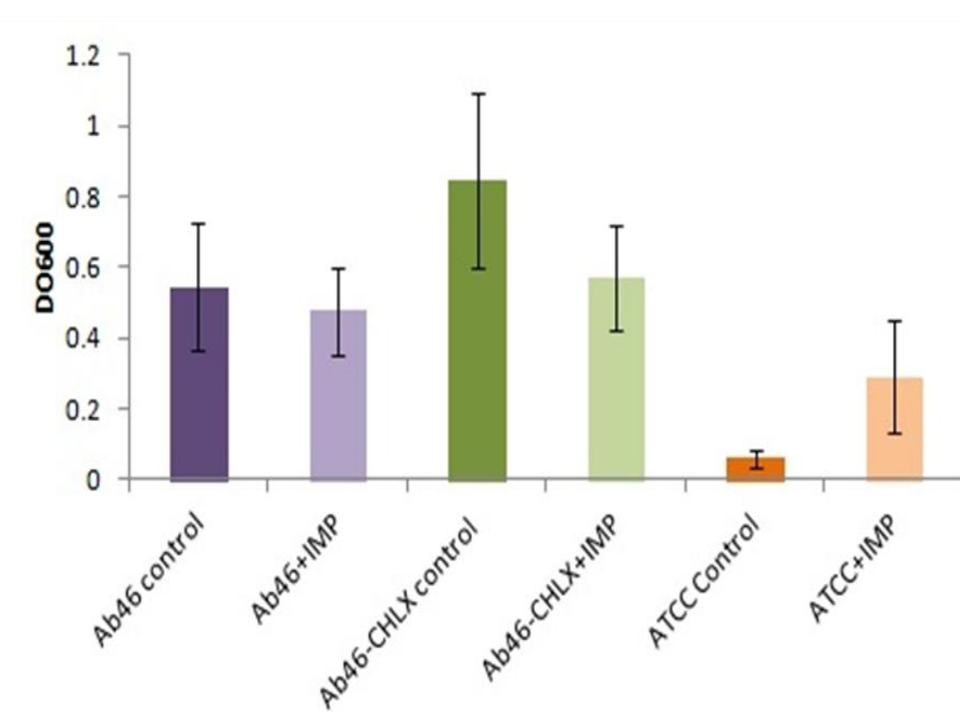
Isolate	SUL	CAZ	IPM	MEM	CIP	GEN	TOB	AMK	MIN	NET	TET	DOX	CST	RIF	TGC
Ab46	64	>128	64	32	32	2	32	32	<0.5	>64	>64	16	<0.5	1	0.5
Ab46 exposed to CHX	32	>128	64	32	32	2	32	16	<0.5	64	>64	16	<0.5	1	0.5

b)

Strain	MIC before CHLX	MIC after CHLX
Ab46	78mg/L	39mg/L



-Figure 1. Bacterial Killing curves. Growth curves of Ab46 and ATCC strains in presence of CHLX and Imipenem.



-Figure 2. Ab46+CHX vs Ab46 strain biofilm. Biofilm quantification experiment in presence of Imipenem.

-Table 2. Transcriptomic analysis in presence of CHLX (Ab46 vs Ab46+CHLX)

Genbank (protein ID)	Function	FoldChange (DESeq2)	FoldChange (EdgeR)	Systems	Rizhome
ODA53993.1	AdeA protein	6.933753475	6.982635042	AdeABC system (RND type)	RESISTOME
ODA53994.1	AdeB protein	6.149907892	6.175526694		
ODA53995.1	AdeC protein	4.257153566	4.270842036		
ODA55718.1	Tetracycline resistance protein	6.119321647	6.133494454	MFS system	RESISTOME
ODA54617.1	Arsenite efflux pump	5.377292457	7.227172031	ACR3 system	
ODA56577.1	Aminoglycoside phosphotransferase	3.498098186	3.528728206	APT family	
ODA54814.1	Chlorhexidine efflux pump	3.605781331	3.649808635	AceI system	RESISTOME
ODA56167.1	MFS transporter	5.265550668	7.054151044	MFS system	
ODA53764.1	Carbapenem-hydrolyzing oxacillinase	12.16763575	14.92175121	AbATCC329p/pMCCU3*	
ODA53763.1	DNA replication protein A	8.975633873	11.30715263		TOLEROME
ODA53762.1	RepB family plasmid replication initiator protein	5.273985066	5.329333593		
ODA54084.1	CsuA protein	3.511019975	3.547062538	CsuABCDE/Biofilm	
ODA54083.1	CsuB protein	3.199749378	3.259685195		TOLEROME
ODA54082.1	CsuC protein	2.575094974	2.584527435		
ODA54081.1	CsuD protein	2.810613341	2.819199271		
ODA54080.1	CsuE protein	2.782552791	2.791313686		TOLEROME
ODA53940.1	Cytochrome b	2.037734523	2.053934504	Stress oxidative (ROS)	
ODA57053.1	Cytochrome bd biosynthesis protein; sodium/proline symporter	2.173049691	2.184371809		
ODA56663.1	Cytochrome bd biosynthesis protein	2.405101873	2.428897655		TOLEROME
ODA56171.1	Cytochrome d ubiquinol oxidase subunit I	10.75708444	13.32903693		
ODA56172.1	Cytochrome d ubiquinol oxidase subunit II	10.35093438	12.86380541		
ODA54604.1	Taurine ABC transporter substrate-binding	10.07652823	12.56398102	Electron transport	TOLEROME
ODA54605.1	Taurine transporter ATP-binding subunit (TauB)	9.758316312	12.21616998		
ODA54606.1	Taurine ABC transporter permease (TauC)	8.966908008	11.30350134		
ODA54607.1	Taurine dioxygenase (TauD)	10.85324686	13.44475271		
ODA55153.1	Hypothetical protein	-6.486154998	-6.530626555	Stringent response	TOLEROME
ODA54592.1	DNA polymerase I	0.932475218	0.929842277		
ODA54625.1	DNA polymerase III subunit alpha	0.931688577	0.929428817		
ODA54730.1	Response regulator	-1.77207536	-1.816317901		TOLEROME
ODA55878.1	50S ribosomal protein L17	0.500078184	0.506140675		
ODA55763.1	RNA polymerase subunit omega	0.438241011	0.436148678		
ODA55654.1	50S ribosomal protein L7/L12	0.582523178	0.580921263		TOLEROME
ODA55933.1	ATP synthase subunit beta	-0.523115918	-0.531876133	Oxidative phosphorylation	
ODA55935.1	ATP synthase subunit alpha	-0.570647356	-0.579254835		
ODA54585.1	Transcription termination factor Rho	0.422390483	0.418615357		TOLEROME
ODA54613.1	Amidohydrolase	8.935636763	11.24329488	Putative antibiotic synthesis	
ODA56169.1	Monooxygenase	8.378615347	10.59873534		
ODA56602.1	SnoL-like domain protein polyketide cyclase	3.732901727	3.821816012		TOLEROME
ODA56597.1	Antibiotic biosynthesis monooxygenase	3.010471156	3.030653334		
ODA53944.1	P-hydroxyphenylacetate hydroxylase C1-reductase component	2.167035978	2.180578541		
	Cyclohexanone monooxygenase	2.122028003	2.134654661		

1 *AbkA/AbkB antitoxin-toxin proteins showed a RE (Relative Expression) of 1.88/3.05 by RT-PCR.
2 *AbaI and AbaR had a RE of 5.69 and 5.46, respectively by RT-PCR, which indicated the Quorum Sensing activation.

Conclusions:

This is the first study where important mechanisms of bacterial persistence/tolerance under chlorhexidine pressure have been described in *Acinetobacter baumannii* ST-2 clinical isolation (harbouring AbkA/AbkB toxin-antitoxin system and OXA 24 β-lactamase). The study of these mechanisms is the key to fight against the development of microbial resistance.

References:
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3-Luo, L. M., et al. (2015). BMC Microbiol 15: 62.
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