PBP2, PBP2a and PBP4 Clone-specific Polymorphism is not Associated to Ceftaroline Susceptibility in Chilean Clinical Isolates of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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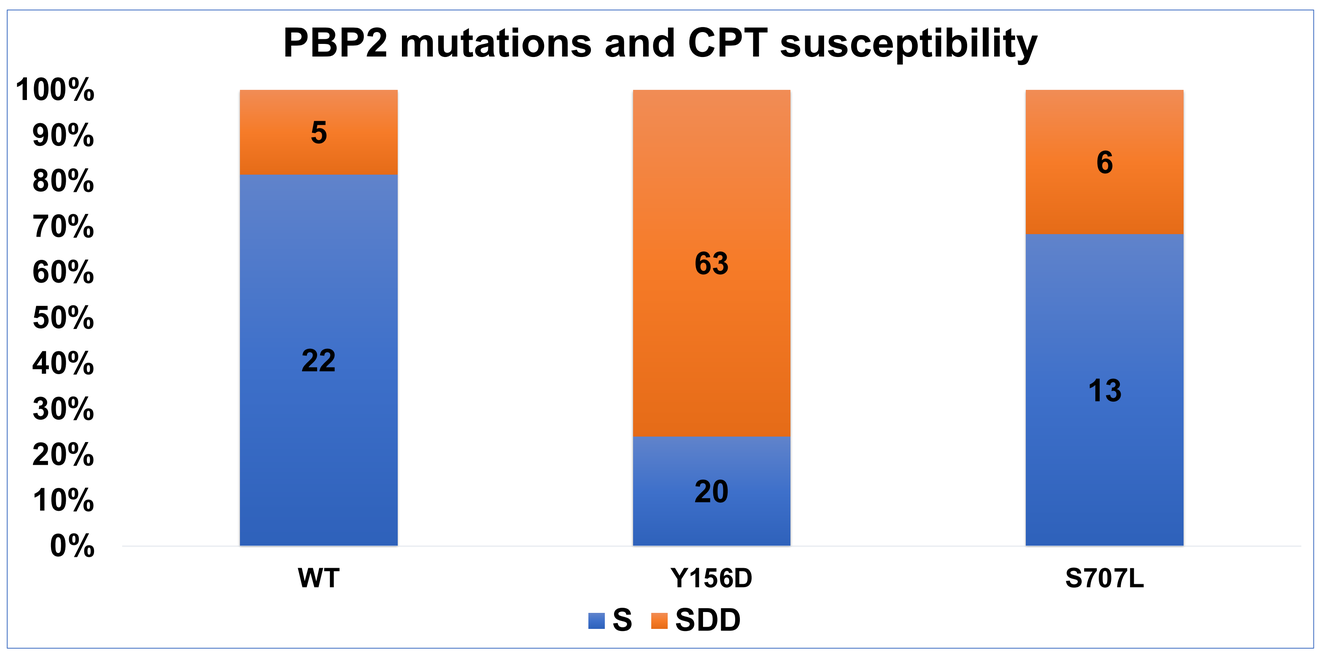
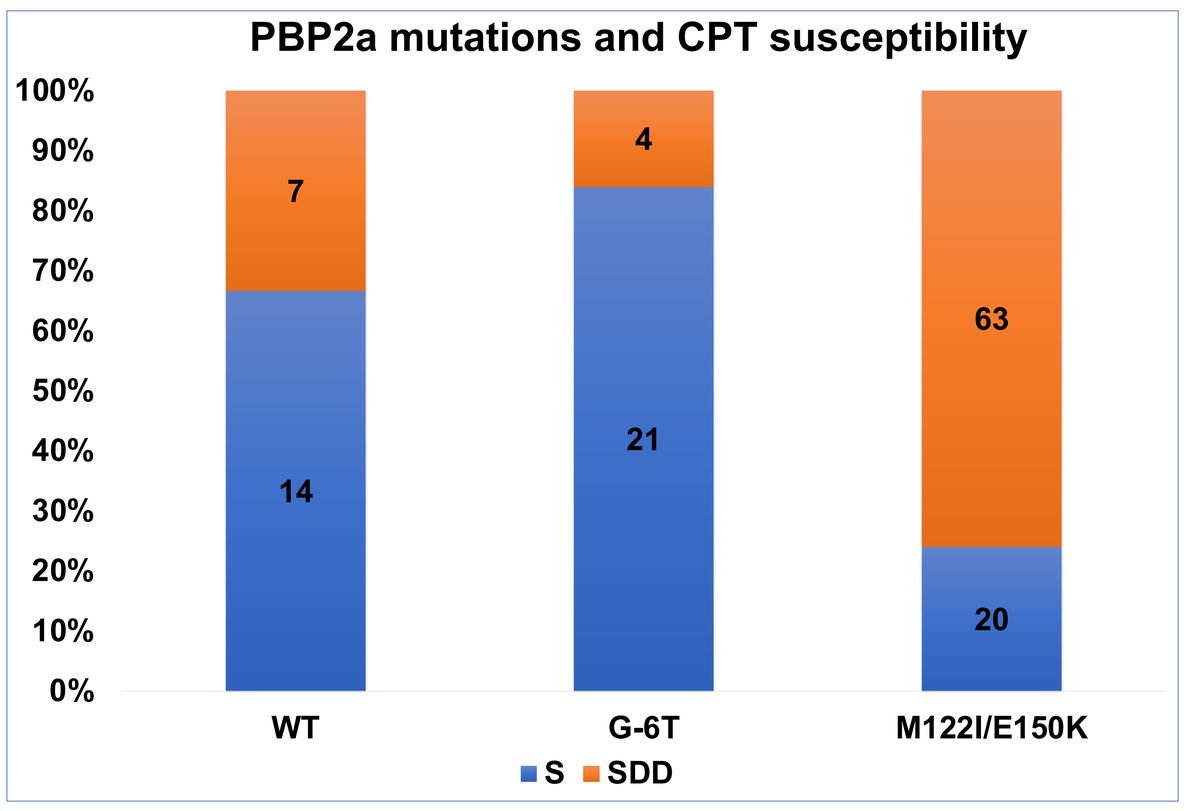
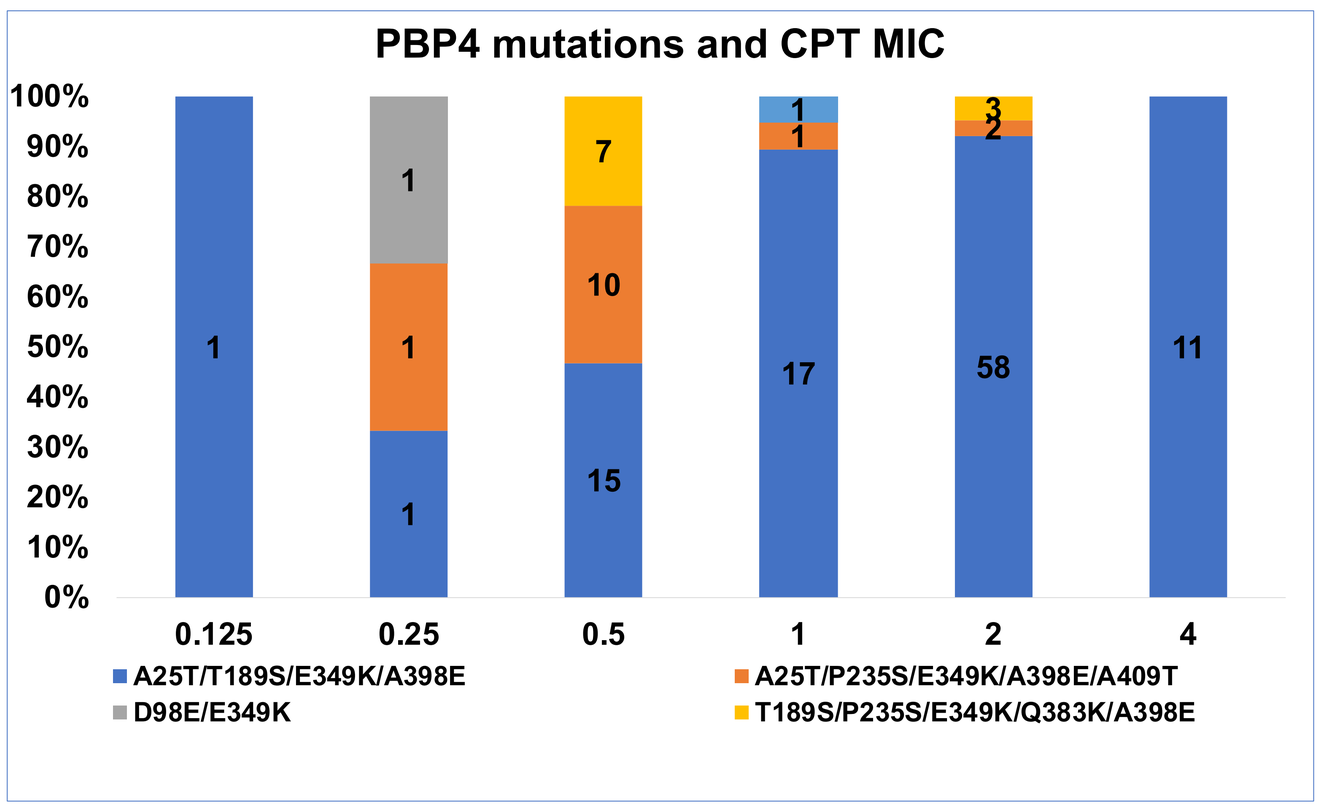
Background: Ceftaroline (CPT) is a new cephalosporin, with activity against MRSA by inhibiting PBP2a. Recent data raised concerns regarding CPT resistance. Previously ,mutations in the Non-Penicillin-binding domain and in the Transpeptidase domain of PBP2a associated to high resistance to CPT, were described. In 2018, Lee *et al.,* reported that the accumulation of mutations in PBP2a resulted in the elevations of the minimal inhibitory concentration (MIC) of CPT. In 2011 Flamm *et al.,* reported high non-susceptibility rate of CPT in Chile. However, the mutational landscape of PBPs in clinical MRSA isolates from Chile has not been assessed. In this study our aim was to identify mutations in PBP2, PBP2a and PBP4 in clinical MRSA isolates and compare them to CPT susceptibility obtained by broth microdilution (BMD).

Methods: We analyzed 180 clinical MRSA isolates collected from 2000-2018 in Santiago, Chile. Identification was confirmed by MALDI-TOF. Cefoxitin-disk diffusion test was performed for methicillin resistance confirmation. Susceptibility to CPT was performed by BMD at a centralized lab and following CLSI-2019 guidance. Whole genome sequencing was performed for all the isolates and the mutational status of PBPs was determined using reference sequences for PBP2 (AGY89563.1), PBP2a (NG_047938.1) and PBP4 (X91786.1).

Results: The MIC_{50}/MIC_{90} by BMD was 2/2µg/dL; only 71 (39%) isolates had an MIC ≤1µg/mL (CPT-susceptible) (table 1). All the isolates were MRSA confirmed by cefoxitin-disk and carried *mecA* gene. Most of the isolates belonged to ST5/SCCmecI (70%, 126/180), ST105/SCCmecII (10%, 18/180) and ST8/SCCmecIV (5%, 9/180). Several mutations were found in PBP2 (Y156D and S707L), PBP2a (g-6t, M122I and E150K) and PBP4 (T25A, D98E, T189S, L234H, P235S, Q383K and T409A) (table 1). Clone-specific polymorphism for PBPs mutations was found. No associations between the number of mutations in PBP2a or presence of mutations in other PBPs and CPT MIC was found.







PBPs mutations compared to CPT MICs by MLST and SCCmec

MLST	SCCmec	Mutational profile			MIC					
		PBP2	PBP2a	PBP4	0.125	0.25	0.5	1	2	4
ST 5	SCCmec I	Y156D	M122I/E150K	T189S, L234H, T409A	1		5	22	86	12
ST 5	SCCmec IV	-	g-6t	T189S, L234H, T409A		1				
ST 105	SCCmec II	S707L	-	T189S, L234H, T409A		1	8	3	3	3
ST 225	SCCmec II	S707L	-	T189S, L234H, T409A			1	1		
ST 125	SCCmec IV		g-6t	T189S, T409A			1	1		
ST 72	SCCmec IV	-	g-6t	T25A, T189S, P235S, Q383K, T409A			4		2	
ST 72	SCCmec VI	-	g-6t	T25A, T189S, P235S, Q383K, T409A			3		1	
ST 1472	SCCmec IV	-	g-6t	T25A, L234H, T409A			1			
ST 22	SCCmec IV		g-6t	T25A, D98E, E398A, T409A		1				
ST 2802	SSCmec IV	-	g-6t	P235S			2			
ST 2039	SCCmec III	-	-	P235S				1		
ST 923	SCCmec IV	-	g-6t	P235S		1	1			
ST 8	SCCmec IV	-	g-6t	L234H			8		1	
ST 239	SCCmec III	-	-	L234H				3	1	
ST UN	SCCmec IV	-	g-6t	L234H			1			

Conclusion: Despite previous described mutations in PBPs were related to CPT resistance, in our Chilean isolates resistance to CPT was not associated to the clone-specific mutational profile. These findings suggest that the MIC to CPT is a clone specific event poorly related to PBPs mutations.