

# In vitro multisite study of vancomycin and daptomycin MIC results and tolerance of contemporary *Staphylococcus aureus* from Spain

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## Abstract

**Objective:** The purpose of this study was to determine daptomycin and vancomycin MIC and MBC results for recent *Staphylococcus aureus* clinical isolates at various medical centers in Spain. **Methods:** A total of 6 medical centers from 4 distinct cities in Spain tested 628 contemporary, clinically-significant isolates MRSA (n=516) and MSSA (n=112) isolates from various specimen sources. All isolates were tested to determine MIC and MBC using dried broth microdilution panels containing daptomycin and vancomycin (TREK Diagnostics, West Sussex, England). With exception of dried panels, MIC procedures followed EUCAST guidelines and MBC procedures followed CLSI and ASM guidelines. MIC quality control was performed with *S.aureus* ATCC 29213. MIC interpretative criteria were based on EUCAST clinical breakpoints. No testing was performed to detect hVISA. **Results:** The daptomycin MIC<sub>50/90</sub> for all *S. aureus* was 0.5 mg/L, the range of MIC's was 0.12-2 mg/L (MIC for 3 MRSA was 2 mg/L). The vancomycin MIC<sub>50/90</sub> for all *S. aureus* was 1 mg/L, the range of MIC's was 0.25-2 mg/L (MIC for 17 MRSA and 3 MSSA was 2 mg/L). The percentage of vancomycin tolerant strains for all sites was 6.8% (43/628), 86% of which were MRSA. In comparison, 0.3% (2/628) of strains (both MRSA) were considered daptomycin tolerant (See Table).

Table: Daptomycin and Vancomycin MIC Summary and Vancomycin Tolerance by Testing Site

City	n MRSA/ MSSA	MIC mg/L				% Vancomycin Tolerant*
		Daptomycin 50% 90%	Vancomycin 50% 90%			
Madrid	84/0	0.5 1	0.5 1			15.5
Barcelona	136/93	0.5 0.5	1 1			6.1
Barcelona	80/0	0.25 0.5	0.5 1			11.25
Donostia	98/19	0.5 0.5	1 1			0
Madrid	75/0	0.5 0.5	1 1			6.7
Palma de Mallorca	43/0	0.5 0.5	1 1			4.6
<b>All Sites</b>	<b>516/112</b>	<b>0.5 0.5</b>	<b>1 1</b>			<b>6.8</b>

\*Tolerance defined as MBC/MIC ratio ≥ 16

**Conclusion:** There were no VISA/VRSA strains detected and >99% were susceptible to daptomycin. The higher level of vancomycin tolerant strains compared to daptomycin suggests superior *in vitro* bactericidal activity of daptomycin.

## Methods

**Introduction:** Vancomycin is the standard therapy for MRSA and tolerance to vancomycin has been described among *S. aureus* isolates. It has been suggested that infections caused by vancomycin-tolerant strains may be more difficult to treat, especially when they cause endocarditis or infections in immunocompromised patients. The purpose of this study was to determine daptomycin and vancomycin MIC and MBC results for recent *Staphylococcus aureus* clinical isolates at various medical centers in Spain

**Isolates:** 112 MSSA and 517 MRSA, consisting of clinical isolates that were selected by six Spanish sites; no collection criteria was specified and was biased to include a majority of MRSA. In addition, *S. aureus* (ATCC 29213) was tested by each site for quality control.

### Testing sites:

No.	Institution	No.	Institution
1	Hospital Gregorio Marañon	4	Hospital Donostia
2	Hospital Clinic Barcelona	7	Hospital Universitario Ramon y Cajal
3	Hospital Universitari de Bellvitge	9	Hospital Son Dureta Palma de Mallorca

**MIC Method:** Each isolate was tested one time by broth microdilution according to EUCAST method with exception of using Sensititre dried panels (Trek Diagnostics, E. Grinstead, UK) (1).

**MBC Method:** The CLSI M26A bactericidal method was used with 100 µL transfer (all of well contents from the MIC panel) to plated media for colony counting determination (2-3). The MBC was determined to be the lowest concentration that was bactericidal to ≥99.9% of the original inoculum. Tolerance for this study was defined as an MBC/MIC ratio of ≥16.

Figure: Daptomycin (DAP) and Vancomycin (VAN) MIC and MBC distributions (Number at each concentration for all sites, n=628)

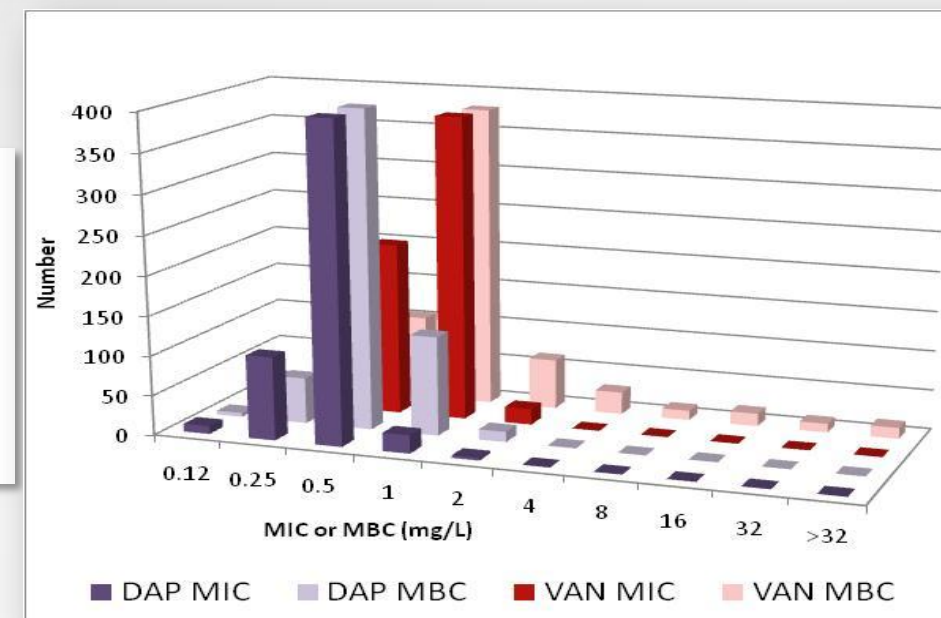


Table 1: Daptomycin MIC and MBC distribution (mg/L) by Site

Site No.	City	Total N	MIC mg/L								MBC/MIC ≥16 (N)		
			0.12	0.25	0.5	1	2	4	32	>32			
1	Madrid	84	MIC		11	62	9	2					0
			MBC			7	66	11					
2	Barcelona	229	MIC		35	193	1						0
			MBC		17	182	30						
3	Barcelona	80	MIC	9	33	30	7	1					1
			MBC	4	29	34	11	2					
4	Donostia	117	MIC		5	112							0
			MBC		1	109	7						
7	Madrid	75	MIC	1	17	54	3						1
			MBC	1	9	57	6		1	1			
9	Palma de Mallorca	43	MIC		4	36	3						0
			MBC		2	36	5						
<b>All Sites</b>		<b>628</b>	<b>MIC</b>	<b>10</b>	<b>105</b>	<b>487</b>	<b>23</b>	<b>3</b>					<b>2</b>
			<b>MBC</b>	<b>5</b>	<b>58</b>	<b>425</b>	<b>125</b>	<b>13</b>	<b>1</b>	<b>1</b>			

Table 2: Vancomycin MIC and MBC distribution (mg/L) by Site

Site No.	City	Total N	MIC mg/L										MBC/MIC ≥16 (N)		
			0.25	0.5	1	2	4	8	16	32	>32				
1	Madrid	84	MIC	8	59	12	5								13
			MBC		1	42	24	3	2	2	2	8			
2	Barcelona	229	MIC	1	67	153	8							14	
			MBC		49	123	21	19	3	7	7				
3	Barcelona	80	MIC		49	30	1							9	
			MBC		30	36	3	1	1	6*	1**	2			
4	Donostia	117	MIC		19	95	3							0	
			MBC		4	102	9	2							
7	Madrid	75	MIC		5	67	3							5	
			MBC		4	53	5	2	6		1	4			
9	Palma de Mallorca	43	MIC		18	25								2	
			MBC		17	23	1	1		1					
<b>All Sites</b>		<b>628</b>	<b>MIC</b>	<b>9</b>	<b>217</b>	<b>382</b>	<b>20</b>							<b>43</b>	
			<b>MBC</b>		<b>105</b>	<b>379</b>	<b>63</b>	<b>28</b>	<b>12</b>	<b>16</b>	<b>11</b>	<b>14</b>			

\*Includes 4 strains with MBCs ≥16 mg/L  
\*\*MBC ≥32 mg/L

Table 1 and 2 Color Legend MIC or MBC: 50 90 50 & 90

Table 3: Daptomycin (DAP) and Vancomycin (VAN) MIC and MBC (mg/L) for *S. aureus*\* with vancomycin MBC/MIC ≥16

Site No.	Strain No.	Daptomycin		Vancomycin	
		MIC	MBC	MIC	MBC
Site 1: Madrid	1	0.25	1	0.5	64
	2	0.25	1	0.25	8
	5	0.5	1	0.5	32
	8	0.25	1	0.5	8
	11	0.25	0.5	0.25	16
	12	0.25	0.5	0.5	64
	19	2	2	2	>64
	22	0.5	1	0.5	>64
	33	1	1	1	>32
	35	0.5	1	0.5	32
	39	0.5	1	0.5	>32
	76	0.5	1	0.5	>32
	83	0.5	1	0.5	16
Site 2: Barcelona	9	0.5	0.5	1	32
	10	0.5	0.5	1	16
	12	0.5	0.5	1	16
	23	0.5	0.5	1	16
	32	0.5	1	1	16
	54	0.5	0.5	1	16
	86	0.5	1	1	16
	88	0.5	0.5	1	32
	118	0.5	0.5	0.5	>16
	175	0.5	1	0.5	>16
	188	0.5	0.5	1	>16
	192	0.5	0.5	1	>16
	193	0.5	0.5	1	>16
210	0.5	0.5	1	16	
Site 3: Barcelona	3	0.125	2	0.5	8
	4	0.12	0.5	0.5	>64
	7	0.12	0.5	0.5	16
	8	0.5	0.5	0.5	>64
	27	0.5	0.5	1	≥32
	31	0.12	0.12	0.5	≥16
Site 7: Madrid	37	0.5	0.5	0.5	≥16
	43	0.25	0.5	0.5	≥16
	45	0.5	0.5	0.5	≥16
	17	1	32	2	64
	38	0.5	0.5	1	64
Site 9: Palma de Mallorca	39	0.5	0.5	1	64
	40	0.5	1	1	64
	41	0.5	0.5	1	32
All Sites	17	0.5	0.5	1	16
	18	0.5	0.5	0.5	8
		MIC <sub>50/90</sub> = 0.5/0.5		MIC <sub>50/90</sub> = 0.5/1	

\*37 = MRSA. 6 = MSSA (strain no. in purple font)

## Results

### DAPTOMYCIN

- The range of MICs among 628 *S. aureus* was 0.12-2 mg/L
- The range of MBCs was 0.12-32 mg/L. A daptomycin MBC >4 mg/L have not previously been reported.(4)
- The MIC<sub>50/90</sub> was 0.5 mg/L
- The MBC<sub>50</sub> and MBC<sub>90</sub> were 0.5 and 1 mg/L, respectively.
- 3 strains had daptomycin MICs of 2 mg/L (non-susceptible)
- Tolerance was detected in 2/628 strains from 2 different sites.

### VANCOMYCIN

- The range of MICs among 628 *S. aureus* was 0.25-2 mg/L
- The range of MBCs was 0.5->32 mg/L
- The MIC<sub>50/90</sub> was 1 mg/L
- The MBC<sub>50</sub> and MBC<sub>90</sub> were 1 and 4 mg/L, respectively.
- No VISA were found and hVISA testing was not performed
- Tolerance was detected in 5 of 6 Spanish sites at rates of 4.6%-15.5%

## Conclusions

- The higher level of vancomycin tolerant strains detected in this study compared to daptomycin suggests superior *in vitro* bactericidal activity of daptomycin.
- This is the first known report of daptomycin tolerant *S. aureus*. Confirmatory testing of both vancomycin and daptomycin by killing curves and by molecular studies for detection of autolysis is on going.

## References

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