Vancomycin Hetero-resistance has a Small but Significant Effect on the Daptomycin Minimum Inhibitory Concentration of *Staphylococcus aureus*

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J.M. Bell, L.J. Walters, J.D. Turnidge, and R.N. Jones

Women's and Children's Hospital, Adelaide, Australia; JMI laboratories, North Liberty, Iowa.

Jan Bell
email: jan.bell@cywhs.sa.gov.au

Abstract

Background: There are increasing concerns that reduced susceptibility to vancomycin (VAN) may affect the activity of daptomycin (DAP) in *Staphylococcus aureus*.

Methods: Oxacillin-resistant *S. aureus* (n=119) were selected from seven countries as part of the SENTRY Surveillance, Asia Pacific Region. The following parameters were measured; minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to VAN, teicoplanin (TEI) and DAP; and hVISA phenotype screening using the macro ETest method. Tolerance was defined as a MBC/MIC ratio ≥ 32.

Results: There were 64 isolates with a hVISA phenotype. Three strains demonstrated VAN tolerance and 28 strains, including the 3 VAN tolerant strains, demonstrated TEI tolerance. The geometric mean of hVISA phenotypes was 0.28 vs 0.44 for non hVISA phenotypes (P = 0.003), on an unpaired t-test. There was borderline correlation between TEI tolerance and DAP MICs (P = 0.06). There was no correlation between hVISA phenotype, glycopeptide tolerance or DAP MBC/MIC ratio (all P values > 0.1). Four of 64 (6%) hVISA strains had DAP MIC = 2 (intermediate); while none of thee 55 non-hVISAs had elevated DAP MICs.

Conclusion: S. aureus DAP MICs are affected by the presence of hetero-resistance to vancomycin and possibly by vancomycin tolerance.

Introduction

Daptomycin is a cyclic lipopeptide approved for the treatment of complicated skin and skin structure infections. There are increasing concerns that reduced susceptibility to vancomycin may affect the activity of DAP in *S. aureus*.

There are increasing concerns that reduced susceptibility to vancomycin (VAN) may affect the activity of daptomycin (DAP) in *Staphylococcus aureus*. [1]

The aim of this study was to evaluate the MIC and MBC values when testing daptomycin, vancomycin and teicoplanin against *S. aureus* strains collected in the Asia-Pacific region

Methods

Isolates

A total of 119 oxacillin-resistant *S. aureus* isolates were examined as indicated in Table 1. Isolates from China were collected during 1999; all remaining isolates were collected between 2002 and 2004. ATCC 29213 *S. aureus* and ATCC 29212 *Enterococcus faecalis* were used as control strains throughout the study

Bactericidal Activity

Minimum inhibitory concentration (MIC) values were determined by broth microdilution according to CLSI guidelines [2, 3] for daptomycin (128 - 0.06 mg/L), vancomycin and teicoplanin (512 - 0.25 mg/L) and oxacillin (128 - 0.12 mg/L). For daptomycin MICs, Mueller-Hinton Broth (MHB) was supplemented with 50 mg/L calcium, and for oxacillin, MHB was supplemented with 2% NaCl.

Minimum bactericidal concentration (MBC) values were assessed for daptomycin, vancomycin and teicoplanin by plating the entire volume of broth (100 μl) from the broth microdilution MIC well and from those $\log_2$ dilutions above the MIC for each organism onto blood agar plates. Quantitative colony counts were performed on the starting inoculum at the time the MIC test was performed. The lowest concentration of antimicrobial agent that kills ≥ 99.9% of the starting test inoculum was defined as the MBC endpoint.[4]

Tolerance was defined as an MBC/MIC ratio of ≥ 32.

hVISA Phenotype

Isolates were screened for hVISA phenotype using the macro Etest method.[5] Any strain with vancomycin and teicoplanin Etest MIC ≥ 8 mg/L or teicoplanin of ≥ 12 mg/L was considered hVISA phenotype. These phenotypes were confirmed using population analysis profiles.[5,6]

Table 1.hVISA Phenotype vs Country

Country	Yes	no	Total
China	8	7	15
Hong Kong	8	13	21
Japan	12	15	27
Korea	22	4	26
Taiwan	9	13	22
South Africa	3		3
Australia	5		5
Total	*67*	*52*	*119*

Results

- The ratio of MBC/MIC ratio for daptomycin did not exceed 2 for any isolate tested
- Daptomycin MICs were slightly higher for the hVISA phenotypes when compared to non-hVISA strains (Table 2). For hVISA phenotypes daptomycin MIC_{90} was 1 mg/L; for non-hVISA phenotypes MIC_{90} = 0.5 mg/L. The geometric mean of hVISA phenotypes was 0.28 vs 0.44 for non hVISA phenotypes (P = 0.003), on an unpaired t-test
- 25% of 67 isolates with hVISA phenotypes gave a daptomycin MBC/MIC ratio of 2, compared to 17% of 52 isolates with non-hVISA phenotypes; this difference was not statistically significant (Table 3)
- Tolerence to either vancomycin (n=3) or teicoplanin (n=28) was common (Table 4). Strains with hVISA phenoptypes were more likely to demonstrate tolerance than non-hVISA phenotypes (27% vs 19%) (Table 5), but the difference was not statistically significant. There was no significant association with tolerance and daptomycin MBC/MIC ratio of 2 (Table 6)

References

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Table 2.MIC and MBC Distributions

	Agent	hVISA	Number of isolates at MIC/MBC (mg/L) of: 0.25	0.5	1	2	4	8	16	32	64	128	256	512
MIC	Daptomycin	no (n=52)	13	35	4									
		Yes (n=67)	6	46	11	4								
	Vancomycin	no (n=52)		8	43	1								
		Yes (n=67)		3	46	17	1							
	Teicoplanin	no (n=52)		11	28	9	4							
		Yes (n=67)		3	18	18	15	13						
MBC	Daptomycin	no (n=52)	7	38	7									
		Yes (n=67)	5	35	20	6	1							
	Vancomycin	no (n=52)		6	36	7	2				1			
		Yes (n=67)		1	28	31	4	1				1	1	
	Teicoplanin	no (n=52)		2	5	13	8	8	5		6	4	1	
		Yes (n=67)			4	5	13	10	6	4	10	7	6	2

Table 3	Daptomycin MBC/MIC Ratio		Total
	1	2	
hVISA	50	17	67
Not hVISA	43	9	52
Total	93	26	119

P=0.3726

Table 5	Tolerance		Total
	Yes	No	
hVISA	18	49	67
Not hVISA	10	42	52
Total	28	91	119

P=0.3875

Table 6	Daptomycin MBC/ MIC Ratio		Total
	1	2	
Tolerance	19	9	28
No tolerance	74	17	91
Total	93	26	119

P=0.1891

Table 4. Distribution of Isolates vs MBC/MIC Ratio

MBC/MIC Ratio	Number of Isolates: Daptomycin No[a]	Daptomycin Yes	Vancomycin No	Vancomycin Yes	Teicoplanin No	Teicoplanin Yes
1	43	50	41	39	8	1
2	9	17	8	24	13	15
4			2	2	10	12
8					6	8
16					5	9
32					2	14
64			1	2	3	1
128					2	3
256					3	

[a] hVISA Screen positive: Yes / no

Conclusion

- **Oxacillin-resistant Staphylococcus aureus daptomycin MICs are affected by the presence of hetero-resistance to vancomycin and possibly by vancomycin tolerance**