C2-1791 Daptomycin Susceptibility of Staphylococci from Latin America, Australia and New Zealand

QUOTIENT BIORESEARCH

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Abstract

Background: The *in vitro* activity of daptomycin (DAP) was determined against staphylococci collected in 2008/9 from 37 hospitals in Argentina (ARG), Australia (AUS), Brazil (BRA), Mexico (MEX) and New Zealand (NZ).

Methods: 272 methicillin-resistant and susceptible *S. aureus* (MRSA & MSSA) and 256 coagulase-negative staphylococci (CoNS-MR & CoNS-MS) – predominantly *S. epidermidis* (149) were collected. CLSI broth microdilution MIC was determined for daptomycin (DAP), levofloxacin (LEV), ceftriaxone (AXO), linezolid (LZD), vancomycin (VAN), teicoplanin (TEI), erythromycin (ERY), clindamycin (CLI), gentamicin (GEN) and tigecycline (TGC). CLSI breakpoints were used except for TGC (EUCAST breakpoints were used).

Results: MSSA or CoNS-MS percent susceptibility (%S) did not differ by country. MRSA from BRA and MEX had lower %S than those from ARG or AUS to ERY (≤10% compared with (cf) ~40%), CLI (~20% cf ~70%) or LEV (~10% cf ~50%). For CoNS-MR, AUS isolates had higher %S than those from BRA, MEX or ARG to CLI (88% cf 36-51%) ERY (38% cf 11-24%) or LEV (68% cf 16-40%). Two CoNS-MR from BRA were LZD non-susceptible (NS) with MIC ≥32 mg/L. All *S. aureus* were DAP susceptible, but 6 CoNS were DAP NS (MIC = 2 mg/L, overall susceptibility 97.7%) - 3 MS *S. sciuri* (BRA), 1 MS *S. sciuri* (ARG), 1 MR *S. sciuri* (MEX) and 1 MR *S. epidermidis*. The DAP NS *S. sciuri* from BRA & MEX were an identical clone but the isolate from ARG was unique (by PFGE).

Conclusion: DAP showed very good activity against staphylococci (100 %S against SA and 97.7 %S against CoNS) from BRA, ARG, AUS & NZ, in agreement with data from other countries.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and Coagulase-negative staphylococci (CoNS-MR) cause various serious hospital- and community-acquired infections and have become more prevalent and are associated with greater morbidity and mortality than wild-type strains [1, 2].

In recent years linezolid, quinupristin/dalfopristin, tigecycline, and daptomycin have been approved for the treatment of resistant Gram-positive infections. Vancomycin is currently the gold standard treatment for infections caused by *S. aureus*, however, concern is growing over a perceived 'MIC creep' which has been shown to have a profound effect on antimicrobial efficacy [3], patient mortality [4], duration of hospital stay [5] and overall cost of therapy [6].

Daptomycin is the first of a new class of cyclic lipopeptide with a broad spectrum of activity against Gram-positive bacteria, including MRSA, vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) [7].

Daptomycin has a distinct mechanism of action, insertion of its lipophilic tail into the cell membrane causes calcium-dependent efflux of potassium and rapid depolarization ultimately resulting in inhibition of macromolecular synthesis leading to bacterial cell death [8].

Daptomycin is licensed in Europe for treatment of complicated skin and soft tissue infections (cSSTIs) and *S. aureus* bacteraemia (SAB) and right-sided infective endocarditis (RIE).

Material and Methods

The *in vitro* activity of daptomycin and comparator antimicrobials was determined against staphylococci collected in 2008/9 as part of the Novartis Daptomycin Surveillance Study. Thirty-seven hospitals from Argentina, Australia, Brazil, Mexico and New Zealand were invited to submit methicillin-resistant and -susceptible *S. aureus* and coagulase-negative staphylococci (CoNS) isolates to Quotient Bioresearch Ltd. which acted as the central testing laboratory.

Pathogens were isolated at each centre between 1st April 2008 and 31st January 2009 from skin & soft-tissue infection, bacteraemia, endocarditis, osteomyelitis and urinary tract infections. Repeat isolates from the same infection episode were excluded.

In total, 272 methicillin-resistant and susceptible *S. aureus* (MRSA & MSSA) and 256 coagulase-negative staphylococci (CoNS-MR & CoNS-MS) – predominantly *S. epidermidis* (149) were collected. Quotient Bioresearch Ltd. confirmed the identification of all isolates and CLSI broth microdilution MIC was determined for daptomycin (DAP), vancomycin (VAN), teicoplanin (TEI), gentamicin (GEN), ceftriaxone (AXO), levofloxacin (LEV), erythromycin (ERY), tigecycline (TGC), clindamycin (CLI) and linezolid (LZD) by the CLSI broth microdilution method [9] using freeze-dried 96-well panels provided by TREK Diagnostics (East Grinstead, UK).

Susceptibility of staphylococci to daptomycin was defined by the EUCAST (and CLSI) break point of 1mg/L. Susceptibility to all other agents was defined by the CLSI break point [10] except for tigecycline where EUCAST break points were used [11]. Identification of these non-susceptible strains was confirmed by MALDI-ToF mass spectrometry using a Microflex Biotyper (Bruker Daltonik GmbH) and 16s rRNA gene sequencing using an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Warrington, UK [12, 13]. Epidemiological typing of strains with reduced susceptibility to daptomycin was performed by Pulsed Field Gel Electrophoresis (PFGE) digestion using the restriction enzyme *sma*I as previously described [14].

Results

Table 1 - Isolates collected by Country

Pathogen	Resistance	Country (N) ARG	AUS	BRA	MEX	NZ	Grand Total
S. aureus	Methicillin-resistant	42	37	38	34	10	161
	Methicillin-susceptible	23	20	26	36	6	111
All CNS	Methicillin-resistant	35	34	38	40	9	156
	Methicillin-susceptible	26	21	22	24	7	100
S. capitis	Methicillin-resistant	1	2			1	4
	Methicillin-susceptible		1		2	1	4
S. caprae	Methicillin-resistant		1				1
	Methicillin-susceptible			1	1	1	3
S. chromogenes	Methicillin-resistant	1					1
S. cohnii	Methicillin-resistant	1		1			2
	Methicillin-susceptible		1		1		2
S. epidermidis	Methicillin-resistant	24	22	22	28	6	102
	Methicillin-susceptible	15	12	8	11	1	47
S. haemolyticus	Methicillin-resistant	5	4	12	8	1	30
	Methicillin-susceptible		1		2		3
S. hominis	Methicillin-resistant	3	4	1	3	1	12
	Methicillin-susceptible	1	2	2	3		8
S. kloosii	Methicillin-susceptible	2					2
S. lugdenensis	Methicillin-susceptible	3	2	1		1	7
S. saprophyticus	Methicillin-resistant		1				1
	Methicillin-susceptible	3		1	1		5
S. sciuri	Methicillin-resistant				1		1
	Methicillin-susceptible	1		3			4
S. simulans	Methicillin-susceptible	1	1	1	1		4
S. warneri	Methicillin-resistant			2			2
S. warneri	Methicillin-susceptible		1	5	1	3	10

Argentina (ARG), Australia (AUS), Brazil (BRA), Mexico (MEX) and New Zealand (NZ)

Results cont.

Figure 1 - Daptomycin MIC distribution against MRSA (n= 161), MSSA (n=111), CoNS-MR (n=156) and CoNS-MS (n=100)

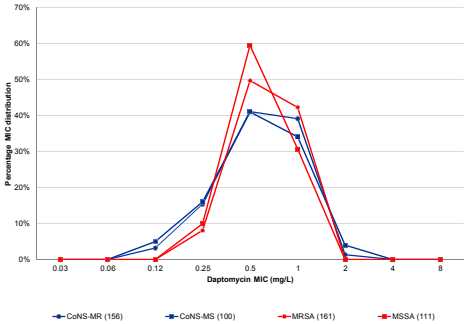


Table 2 - Susceptibility profile for all countries

		AXO	CLI	DAP	ERY	GEN	LEV	LZD	TEI	TGC	VAN
MRSA (161)	% S	-	47.2	100	26.7	68.3	31.1	100	100	100	100
	MIC50/90	64/64	8/8	0.5/1	64/64	0.5/32	8/32	2/4	0.5/1	0.25/0.25	1/1
MSSA (111)	% S	99.1	97.3	100	82.9	93.7	94.6	100	100	100	100
	MIC50/90	4/4	0.12/0.25	0.5/1	0.5/64	0.5/1	0.25/0.5	2/4	0.5/0.5	0.25/0.25	1/1
CoNS-MR (156)	% S	-	54.5	98.7	22.4	35.3	41	98.7	98.1	100	100
	MIC50/90	32/64	0.12/8	0.5/1	64/64	16/32	4/32	1/2	2/8	0.25/0.5	2/2
CoNS-MS (100)	% S	96.0	96.0	96.0	73.0	91.0	92.0	100	97.0	100	100
	MIC50/90	2/8	0.06/0.25	0.5/1	0.25/64	0.06/2	0.25/0.5	1/2	1/8	0.12/0.25	1/2

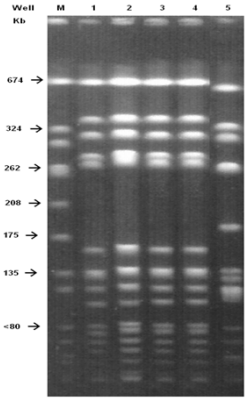
"-" Not applicable

Figure 2 - Gel showing *sma*I macrorestriction patterns of the five isolates of *S. sciuri* from Latin America with non-susceptibility to daptomycin.

Lane M: S. aureus NCTC 8325 (which was used as a size standard);

1: IV355-021010 (Brazil)
2: IV355-021011 (Brazil)
3: IV355-021013 (Brazil)
4: IV355-029008 (Mexico)
5: IV355-015010 (Argentina)

The sizes of the marker fragments are given in Kb.



Results

- Overall susceptibility to daptomycin in *S. aureus* and CoNS was high (100% in *S. aureus* and 97.4% in CoNS).
- Daptomycin percentage susceptibility (%S) for MSSA, CoNS-MS and MRSA did not differ by country. Daptomycin %S for MR-CoNS did vary slightly between countries [Australia (97%), Mexico (97.5%), Brazil (100%) and Argentina (100%).
- MRSA from Brazil and Mexico had lower %S than those from Argentina and Australia to erythromycin (≤10% compared with ~40%), clindamycin (~20% compared with ~70%) and levofloxacin (~10% compared with ~50%). CoNS-MR isolates from Australia had higher %S than those from Brazil, Mexico and Argentina to clindamycin (88% compared with 36-51%), erythromycin (38% compared with 11-24%) and levofloxacin (68% compared with 16-40%).
- Six CoNS [3 MS *S. sciuri* (Brazil), 1 MS *S. sciuri* (Argentina), 1 MR *S. sciuri* (Mexico) and 1 MR *S. epidermidis*] were daptomycin non-susceptible (MIC = 2 mg/L, overall susceptibility 97.7%). The results of the *sma*I restriction show that the three *S. sciuri* isolates from Brazil and the *S. sciuri* isolate from Mexico are an identical clone. The *S. sciuri* strain from Argentina appears unrelated.
- 2 CoNS-MR from Brazil were linezolid non-susceptible with MIC ≥32 mg/L but showed susceptibility to daptomycin (MIC 1 mg/L).

Conclusions

- Daptomycin showed very good activity against staphylococci (100 %S against *S. aureus* and 97.7 %S against coagulase negative staphylococci) from Brazil, Argentina, Australia and New Zealand in agreement with data from other countries.

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