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German Surveillance Study on the In Vitro Activity of Daptomycin against Gram-Positive Cocci

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Revised Abstract

Objectives: Drug resistance (R) in Gram+ bacteria has become an increasing problem over the past 20 years. New antimicrobial agents such as the lipopeptide daptomycin (DAP) have been developed to address this problem. Shortly after the approval of DAP in the EU, a surveillance study was started by a network of 73 laboratories to monitor the susceptibility of Gram+ bacteria to DAP in Germany. The objective of this study was to evaluate the susceptibility of isolates of six clinically important Gram+ species to DAP and other antimicrobial agents.

Methods: A total of 2,645 isolates incl. methicillin-susceptible *Staphylococcus aureus* (MSSA, n=661), methicillin-resistant *S. aureus* (MRSA, n=439), *Staphylococcus epidermidis* (Se, n=425), *Enterococcus faecalis* (Es, n=341), *Enterococcus faecium* (Em, n=324), *Streptococcus pyogenes* (Spy, n=318) and *Streptococcus agalactiae* (Sag, n=137) were tested against DAP, linezolid (LZD), vancomycin (VAN) and other drugs. Primary isolates obtained from hospitalized patients with skin and soft tissue infections, respiratory tract infections, foreign body/catheter infections or sepsis were included. MICs were determined in a central laboratory using the microdilution method according to the German DIN standard. For DAP, the Ca^{2+} concentration of the test solution was adjusted to 50 mg/L. MICs were interpreted by EUCAST criteria, when possible.

Results: Isolates were primarily recovered from skin/wound swabs (64%), blood samples (21%) and respiratory specimens (9%). $MIC_{50/90}$s of DAP for MSSA, MRSA, and Se each were 0.5/1 mg/L. All but one staphylococcal isolates were inhibited by DAP at the breakpoint of 1 mg/L. The remaining isolate had an MIC of 2 mg/L. All staphylococci were susceptible to LZD and VAN. The highest MIC of VAN for MRSA was 2 mg/L. Based on $MIC_{50/90}$s, DAP was up to 4-times more active than LZD or VAN against staphylococci.

Of the Em and Es isolates, 33 (10%) and one (0.3%) were R to VAN, respectively. DAP inhibited all strains at 4 mg/L. One Es isolate was resistant to LZD (MIC 32 mg/L). High-level R to gentamicin (MIC >500 mg/L) was observed in 35%/42% of Es/Em isolates.

DAP, with $MIC_{50/90}$s of 0.125/0.25 mg/L, was more active than LZD (1/1 mg/L) or VAN (0.25/0.5 mg/L) against Spy. Against Sag, DAP (0.5/1 mg/L) was more active than LZD (1/2 mg/L) and comparably active to VAN (0.5/0.5 mg/L). R to erythromycin was detected in 9% of Spy isolates and 23% of Sag isolates.

Conclusion: DAP demonstrated excellent in vitro activity against a large collection of German Gram+ cocci including MRSA and VRE.

Introduction and Purpose

Drug resistance in Gram-positive bacteria has become an increasing problem over the past 20 years (1). New antibacterial agents such as daptomycin (DAP) have been developed to address this problem (2,3). DAP, a cyclic lipopeptide, exerts high in vitro activity against a range of Gram-positive pathogens such as methicillin (oxacillin)-susceptible and methicillin (oxacillin)-resistant *Staphylococcus aureus* (MSSA, MRSA), coagulase-negative staphylococci, enterococci (including vancomycin-resistant strains) and streptococci. It binds to bacterial membranes and causes a rapid depolarization of membrane potential leading to inhibition of protein, DNA, and RNA synthesis. DAP has been demonstrated to exert rapid, concentration-dependent bactericidal activity and a long lasting post-antibiotic effect (3,4).

In January 2006, the European Medicines Agency (EMEA) granted approval to DAP for the treatment of complicated skin and skin-structure infections (cSSTI). In the meantime, DAP has also received approval for the treatment of right-sided infective endocarditis (RIE) due to *S. aureus* and *S. aureus bacteraemia* (SAB) when associated with RIE or with cSSTI (5). Shortly after the first approval, a surveillance study (DAPTOGERM) was started by a network of 73 clinical microbiology laboratories to monitor the susceptibility of Gram-positive pathogens to DAP and other antimicrobial agents in Germany. This report comprises the results for six frequently encountered Gram-positive species.

Methods

Bacterial strains

A total of 2,645 isolates including MSSA (n=661), MRSA (n=439), *Staphylococcus epidermidis* (n=425), *Enterococcus faecalis* (n=341), *Enterococcus faecium* (n=324), and *Streptococcus pyogenes* (n=318) collected during a 8-month period (March to October 2006) were tested against DAP, linezolid (LZD), vancomycin (VAN), ampicillin, oxacillin, penicillin, erythromycin, moxifloxacin, and gentamicin. Primary isolates obtained from hospitalized patients with skin and soft tissue infections, respiratory tract infections, foreign body/catheter infections, or sepsis were included. The number of isolates collected by individual centres ranged between 11 and 44 (median 37).

Table 1: Susceptibility of methicillin-susceptible *Staphylococcus aureus* (n=661)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256					
Oxacillin							546	115									0.5	1	100	-	0
Daptomycin				1	9	99	461	91									0.5	1	100	-	0
Linezolid								25	551	85							2	4	100	-	0
Vancomycin							300	353	8								1	1	100	0	0
Erythromycin							6	261	278	7	2	2	*105*				2	≥ 32	40.4	42.1	17.5
Moxifloxacin			28	358	157	27	5	8	18	48	6	1	*5*				0.063	2	87.0	1.2	11.8
Penicillin		28	117	34	14	22	18	55	69	77	91	75	*61*				2	16	29.2	-	70.8

Table 2: Susceptibility of methicillin-resistant *Staphylococcus aureus* (n=439)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256					
Oxacillin									1		2	26	28	109	177	96	128	≥ 256	0	-	100
Daptomycin				1	6	35	309	87	1								0.5	1	99.8	-	0.2
Linezolid								17	377	45							2	4	100	-	0
Vancomycin							198	221	20								1	1	100	0	0
Erythromycin							2	41	35	2	2	2	*355*				≥ 32	≥ 32	9.8	8.0	82.2
Moxifloxacin				7	3	2	3	5	104	265	44	4	*2*				4	8	3.4	1.1	95.4
Penicillin									7	6	28	183	*215*				16	≥ 32	0	-	100

Table 3: Susceptibility of methicillin-resistant *Staphylococcus epidermidis* (n=425)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256					
Oxacillin							124	6	9	9	19	30	54	85	65	24	32	128	30.6	-	69.4
Daptomycin			**1**		5	62	262	95									0.5	1	100	-	0
Linezolid						3	6	195	220	1							2	2	100	-	0
Vancomycin							8	172	238	7							2	2	100	0	0
Erythromycin						1	10	102	27	1	2	2	*280*				≥ 32	≥ 32	26.6	6.4	67.1
Moxifloxacin			3	41	100	9	25	83	118	11	12	21	*2*				1	4	41.9	19.5	38.6
Penicillin	3	30	8	3	11	16	20	36	35	44	67	113	*39*				8	16	12.9	-	87.1

Table 4: Susceptibility of methicillin-resistant *Enterococcus faecalis* (n=341)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128	≥ 500					
Daptomycin						1	20	82	125	113							2	4	-	-	-
Linezolid							3	80	247	10			1				2	2	99.7	-	0.3
Vancomycin						3	9	212	113	3					1		1	2	99.7	0	0.3
Moxifloxacin				1	1	80	114	9	5	8	19	80	*24*				0.5	16	-	-	-
Ampicillin						2	21	213	76	29							1	2	91.5	8.5	0
Gentamicin																118	-	-	-	-	34.6*

Table 5: Susceptibility of methicillin-resistant *Enterococcus faecium* (n=324)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128	≥ 500					
Daptomycin						1	11	32	141	139							2	4	-	-	-
Linezolid							2	85	232	5							2	2	100	-	0
Vancomycin						4	170	108	6	1	2			1	32		0.5	64	89.2	0.6	10.2
Moxifloxacin						2	9	3	14	8	12	84	*192*				≥ 32	≥ 32	-	-	-
Ampicillin							3	6	5	4	4	3	11	38	250		≥ 128	≥ 128	4.3	2.5	93.2
Gentamicin																136	-	-	-	-	42.0*

Table 6: Susceptibility of methicillin-resistant *Streptococcus pyogenes* (n=318)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256					
Daptomycin			1	62	148	83	24										0.125	0.25	100	-	0
Linezolid			1			5	67	244	1								1	1	100	0	0
Vancomycin					2	185	131										0.25	0.5	100	-	0
Erythromycin		1	4	47	199	38				2	2	8	*17*				0.125	0.25	90.9	0	9.1
Moxifloxacin				12	132	144	30										0.25	0.25	100	0	0
Penicillin	318																≤ 0.008	≤ 0.008	100	0	0

Table 7: Susceptibility of methicillin-resistant *Streptococcus agalactiae* (n=137)

Antibacterial agent	MIC (mg/L)																MIC_{50} (mg/L)	MIC_{90} (mg/L)	%S	%I	%R
	≤ 0.008	0.016	0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256					
Daptomycin					6	60	54	17									0.5	1	100	-	0
Linezolid							3	113	21								1	2	100	0	0
Vancomycin						10	126	1									0.5	0.5	100	-	0
Erythromycin				1	38	67				4	6	2	*19*				0.25	≥ 32	77.4	0	22.6
Moxifloxacin			1		11	114	11										0.25	0.25	100	0	0
Penicillin	3	5	61	67	1												0.031	0.063	100	0	0

Abbreviations: %S, % susceptible strains; %I, % intermidiate strains; %R, % resistant strains
The number in bold represents an isolate inhibited at the lowest concentration tested; numbers in italic comprise isolates with MICs > the highest concentration tested.
*% of strains with high-level resistance to gentamicin

Isolates were identified to the species level by routine methods and then stored on cryo-beads at –70°C. At the end of the collection period, isolates were shipped to a referral laboratory (Antiinfectives Intelligence) on Amies transport media with charcoal (Mastaswab™, Mast Diagnostica, Reinfeld, Germany) for antimicrobial susceptibility testing.

Susceptibility testing

MICs were determined in a central laboratory using the broth microdilution procedure according to the standard of the German DIN (Deutsches Institut für Normung) 58940 guidelines (6). This was done with industrially manufactured trays (Micronaut-S, Merlin GmbH, Bornheim, Germany). Cation-adjusted Mueller-Hinton broth was used as nutrient medium. For testing DAP the medium was supplemented to 50 mg/L of Ca^{2+}. MICs were interpreted by EUCAST criteria, when possible (7). DIN breakpoints were applied to the other drugs (8). The accuracy of susceptibility testing was evaluated by including quality control organisms.

Results

Most isolates were recovered from wound swabs (64%) followed by blood samples (21.3%) and respiratory tract specimens (9%). Comparative activities of DAP against the 2,645 clinical isolates as well as the susceptibility and resistance rates are shown in **Tables 1-7.**

Staphylococci

DAP exhibited excellent in vitro activity against all isolates. It was equally active against MSSA and MRSA. $MIC_{50/90}$s of DAP for MSSA, MRSA and S. *epidermidis* each were 0.5/1 mg/L. All but one isolates were inhibited by DAP at the breakpoint of 1 mg/L (MIC of 2 mg/L for the remaining isolate). LZD and VAN were also active against all staphylococci. However, based on $MIC_{50/90}$s, DAP was up to fourfold more active than LZD or VAN. Rates of resistance to moxifloxacin were 11.8% for MSSA, 95.4% for MRSA and 38.6% for S. *epidermidis*.

Enterococci

Among the 324 *E. faecium* isolates, 33 (10.2%) were resistant to VAN. In contrast, one *E. faecalis* isolate (0.3%) exhibited resistance to VAN. DAP inhibited all strains at 4 mg/L. $MIC_{50/90}$s of DAP for either enterococcal species were 2/4 mg/L. One *E. faecalis* strain was resistant to LZD (MIC 32 mg/L). 34.6% and 42% of *E. faecalis* and *E. faecium* isolates, respectively, exhibited high-level resistance (MIC >500 mg/L) to gentamicin.

Streptococci

DAP, with $MIC_{50/90}$s of 0.125/0.25 mg/L, was more active than LZD (1/1 mg/L) or VAN (0.25/0.5 mg/L) against *S. pyogenes*. The highest MICs of DAP were 0.5 mg/L. Against *S. agalactiae,* DAP (0.5/1 mg/L) was more active than LZD (1/2 mg/L) and comparably active to VAN (0.5/0.5 mg/L). Resistance to erythromycin was detected in 9.1% of *S. pyogenes* isolates and 22.6% of *S. agalactiae* isolates.

Conclusions

- **DAP demonstrated excellent in vitro activity against all six Gram-positive species tested.**
- **All but one staphylococcal isolates were susceptible to DAP.**
- **DAP was equally active against MSSA and MRSA.**
- **All enterococci were inhibited by DAP at 4 mg/L.**
- **All streptococci were susceptible to DAP.**
- **DAP seems to be a promising alternative to current agents for the treatment of Gram-positive bacterial infections.**

References

1. McDonald LC. Trends in antimicrobial resistance in health care-associated pathogens and effect on treatment. Clin Infect Dis 2006; 42: S65-71
2. Rybak MJ. The efficacy and safety of daptomycin: first in a new class of antibiotics for Gram-positive infections. Clin Microb Infect 2006; 12 (suppl. 1): 24-32
3. Carpenter CF, Chambers HF. Daptomycin: another novel agent for treating infections due to drug-resistant gram-positive pathogens. Clin Infect Dis 2004; 38: 994-1000
4. Rybak MJ, Hershberger E, Moldovan T, Grucz RG. In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against staphylococci and enterococci, including vancomycin-intermediate and -resistant strains. Antimicrob Agents Chemother 2000; 44: 1062-6
5. Fowler VG Jr, Boucher HW, Corey GR, et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. N Engl J Med. 2006; 355: 653-65
6. Deutsches Institut für Normung. 2004. DIN 58940. Medical microbiology—susceptibility testing of pathogens to antimicrobial agents. Part 8. Microdilution. General method specific requirements, p. 342-53. In Deutsches Institut für Normung e.V. (ed.), DIN-Taschenbuch 222: Medizinische Mikrobiologie und Immunologie - Diagnostische Verfahren. Beuth-Verlag, Berlin, Germany.
7. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical breakpoints. http://www.srga.org/eucastwt/MICTAB/index.html; last visit of the website on March 28th, 2008
8. Deutsches Institut für Normung. 2004. DIN 58940. Medical microbiology—susceptibility testing of pathogens to antimicrobial agents. Part 4. Evaluation classes of the minimum inhibitory concentration – MIC breakpoints of antibacterial agents, p. 313-23. In Deutsches Institut für Normung e.V. (ed.), DIN-Taschenbuch 222: Medizinische Mikrobiologie und Immunologie - Diagnostische Verfahren. Beuth-Verlag, Berlin, Germany.

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