The Journal of Antibiotics (2012) 65, 505–508

www.nature.com/ja

ORIGINAL ARTICLE

In vitro susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from prosthetic joint infections

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Prosthetic joint infections (PJI) are severe complications in Orthopedics, with *Staphylococcus aureus* and *Staphylococcus epidermidis* being the most commonly isolated pathogens. The variable antimicrobial susceptibility found in these microorganisms, along with the increasing number of methicillin-resistant strains, increases the difficulty of antibiotic selection and makes it necessary to perform individual susceptibility studies to select the optimal antibiotic treatment. The aim of this study was to evaluate the *in vitro* susceptibility pattern of 35 clinical strains isolated from PJI (17 *S. aureus* and 18 *S. epidermidis*) against rifampin, vancomycin, tygecicline, clindamycin, cotrimoxazole, cloxacillin, ciprofloxacin, daptomycin and fosfomycin. *In vitro* susceptibility assays were performed using the broth microdilution method and agar dilution for fosfomycin. MBC was also determined. Tygecicline and daptomycin showed the highest antimicrobial activity with low MIC_{90} values, and no resistant strains were detected. On the other hand, ciprofloxacin and cloxacillin exhibited a poor antimicrobial effect with a high percentage of nonsusceptible strains in both species. Bactericidal activity rates revealed the bacteriostatic behavior of rifampin, tygecicline, cotrimoxazole, fosfomycin and clindamycin, whereas vancomycin and cloxacillin showed species- and strain-dependent behavior. Daptomycin and ciprofloxacin were observed to be efficient bactericidal agents against the tested strains. According to our data, rifampin, tigecycline, daptomycin and fosfomycin showed high *in vitro* activity against most staphylococcal strains isolated from the PJIs tested, although daptomycin seems to be the best alternative to vancomycin therapy.

The Journal of Antibiotics (2012) **65,** 505–508; doi:10.1038/ja.2012.62; published online 1 August 2012

Keywords: infection; joint; prostheses; Staphylococcus; susceptibility

INTRODUCTION

The use of joint prostheses has become an extremely important advance in modern medicine because it has helped many patients to improve their quality of life. Nevertheless, prosthetic joint infection (PJI) is a rare but severe complication related to these procedures.[1,2] It is well known that bacterial infection after prosthesis implantation causes high morbidity and even mortality among the affected patients. Moreover, these infections frequently require prosthesis removal and a prolonged antibiotic treatment to cure the patients and ensure complete recovery.[3,4]

Although a wide range of bacterial species can cause PJI,[5] *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most commonly isolated pathogens from implants, representing about 2/3 of the cases.[6] The increasing number of methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidemidis* (MRSE) pathogens is a matter of special concern, as this greater prevalence increases the difficulty of antibiotic selection.[7] Both MRSA and MRSE strains are also usually resistant to other antibiotics, and although vancomycin remains the elective therapy for these cases, its efficacy has been declining over the last few years.[8,9] For this reason, other antibiotics have recently been considered as alternatives to vancomycin.[10–15] Despite these facts, antimicrobial susceptibility remains extremely variable among these microorganisms, making it necessary to perform individual susceptibility studies for each strain to select the best antibiotic combination for treatment.[16]

The aim of this study was to evaluate the *in vitro* susceptibility pattern of *S. aureus* and *S. epidermidis* strains isolated from retrieved prosthetic joint implants against antibiotics commonly used in the treatment of PJI.

MATERIALS AND METHODS

Bacterial strains

Thirty-five clinical *Staphylococcus* isolates (17 *S. aureus* and 18 *S. epidermidis*) as well as two biofilm-producing collection strains, *S. aureus* 15981

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Received 1 March 2012; revised 19 April 2012; accepted 28 June 2012; published online 1 August 2012

(Valle *et al*.[17]) and *S. epidermidis* ATCC 35984, were included. Clinical strains were isolated from orthopedic devices retrieved from patients with diagnosed PJI (one strain per patient) using a previously described protocol[18] between January 2007 and December 2010. Species identification was performed by using the API Staph System (bioMérieux, France). Three *S. epidermidis* clinical strains were identified as small colony variant strains (SCV). All the strains tested were cultured and kept frozen in skim milk at –80 °C until the experiments were performed.

More details about the tested strains are shown in Table 1.

Antibiotics

Nine antimicrobial agents were selected for susceptibility testing: rifampin, vancomycin, ciprofloxacin, cotrimoxazole, cloxacillin, clindamycin, (Sigma, Munich, Germany), tygecicline (Pfizer, New York, NY, USA), daptomycin (Novartis, Basel, Switzerland) and fosfomycin (ERN, Barcelona, Spain). The antibiotics were prepared according to the instructions published by the Clinical and Laboratory Standards Institute (CLSI)[19] and kept frozen at −20 °C until the experiments were performed.

Susceptibility test

In vitro susceptibility assays were performed using the broth microdilution method as described by the CLSI.[19] In the case of fosfomycin, agar dilution was used together with broth microdilution. A calcium supplement ($CaCl_2$ 50 mg l^{-1} of final concentration) was added for daptomycin *in vitro* susceptibility testing. Fosfomycin was tested at the dilution range of 0.06–128 mg l^{-1}; vancomycin, cotrimoxazole, cloxacillin and daptomycin from 0.015 to 32 mg l^{-1}; tygecicline, clindamycin and ciprofloxacin from 0.004 to 8 mg l^{-1}; and rifampin from 0.001 to 4 mg l^{-1}. For MIC determinations, plates were incubated for 24 h, and for the three *S. epidermidis* SCV strains, the incubation time was extended to 48 h. MIC_{50}, MIC_{90} and antibiotic non-susceptibility rates were calculated attending to the susceptibility breakpoints published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI[19,20]

Table 1 Bacterial strains

S. aureus			*S. epidermidis*		
Strain	*Type*	*Source of isolation*	*Strain*	*Type*	*Source of isolation*
15981	Collection	—	ATCC 35984	Collection	—
P1	Clinical	OS	P6.5	Clinical	NS
P2	Clinical	HPC	P23.2	Clinical	HPC
P4	Clinical	HPC	P33.1	Clinical	HPC
P18	Clinical	HPC	P53B	Clinical	HPC
P19	Clinical	HPC	P55	Clinical	HPC
P41	Clinical	OS	P61.T1	Clinical	NS
P61.T3	Clinical	NS	P61.T2	Clinical	NS
P61.T4	Clinical	NS	P74	Clinical	HPC
P62.A	Clinical	OS	P101	Clinical	HPC
P68	Clinical	OS	P146.G	Clinical	NS
P82.1	Clinical	OS	P146.B	Clinical	NS
P95	Clinical	NS	P194	Clinical	NS
P104.1	Clinical	OS	P289.1	Clinical	HPC
P112	Clinical	OS	P289.2	Clinical	HPC
P138	Clinical	HPC	P289.3	Clinical	HPC
P251	Clinical	NS	P223	Clinical	HPC
P272.1	Clinical	KPC	P236	Clinical	KPC
			P281	Clinical	KPC

Abbreviations: HPC, hip prostheses components; KPC, knee prostheses components; NS, nails and screws; OS, osteosynthesis materials.

MBC was calculated by colony counting in trypcase soy agar + 5% sheep blood plates after direct plating of the wells-content without visible microbial growth and interpreted according to an internationally accepted definition.[21]

RESULTS

Table 2 shows the antibiotic susceptibility assay results: MIC_{50} and MIC_{90} (mg l^{-1}) as well as the percentage of nonsusceptible bacterial strains.

Tygecicline and daptomycin showed the highest levels of antimicrobial activity with low MIC_{90} values (0.5 and 1 mg l^{-1} for *S. aureus* and 0.25 and 1 mg l^{-1} for *S. epidermidis*, respectively) and no resistant strains were detected.

Fosfomycin also exhibited substantial activity against both bacterial species, with only one *S. aureus* nonsusceptible strain (5.56% of the total of *S. aureus*). Rifampin and vancomycin showed high activity, with low rates of nonsusceptibility. Cotrimoxazole and clindamycin showed higher activity for *S. aureus* than *S. epidermidis* (5.56 and 11.11%, respectively).

Ciprofloxacin and cloxacillin exhibited a poor antimicrobial effect with a high percentage of nonsusceptible strains in both bacterial species. The high number of MRSA strains in this series is especially remarkable, and was also correlated with *S. aureus* ciprofloxacin nonsusceptibility.

On the other hand, bactericidal activity rates (also shown in Table 2) revealed the bacteriostatic behavior of rifampin, tygecicline, cotrimoxazole, fosfomycin and clindamycin for most strains.

Vancomycin showed a higher bactericidal effect against *S. epidermidis* than against *S. aureus* (94.74% vs 55.56%, respectively), showing strain-dependent behavior. This strain-dependent effect appears also for cloxacillin (50% in both species).

The only two antibiotics that showed bactericidal activity for most strains of both species were ciprofloxacin and daptomycin, both with similar bactericidal activity rates.

DISCUSSION

Treatment of PJI caused by *S. aureus* and *S. epidermidis* is still a challenge because of the variable behavior that these microorganisms exhibit against the different antibiotics available and the increasing number of multidrug-resistant strains seen over recent years.[22] Efficacy in both diagnosis and treatment of such staphylococcal PJIs could be hampered by the emergence of SCV strains or methicillin-resistant strains (especially MRSA), as these are the two most relevant problems.[23,24] SCV strains can remain undetected if incubation is not prolonged, and this has been recommended for the management of patients with clinical signs and symptoms of infection.[25,26] Moreover, infection by SCV strains should be considered a possibility if a poor response to treatment is detected, as previously reported in PJIs caused by *S. aureus*.[27] The three tested SCV *S. epidermidis* strains not only required a minimum period of 2 days for growth in conventional media cultures, but also for MIC and MBC determinations, which required a modification of the established microbiological protocols.

In our study, we have detected an elevated number of MRSA (66.67% of *S. aureus* strains), a fact that confirms the previously reported high prevalence of MRSA.[28] On the other hand, all the MRSA strains tested exhibited an identical resistance phenotype, which is the commonest one seen in our hospital in recent years (data not shown) and has been previously characterized in Spanish isolates.[29] This finding suggests that the availability of antimicrobials which are active against MRSA has been drastically reduced. Likewise, a high cloxacillin nonsusceptibility rate was detected among the *S. epidermidis* strains (68.42%).

Table 2 Results of susceptibility studies: MIC_{50}, MIC_{90} ($mg\,l^{-1}$), nonsusceptibility rate and percentage of bactericidal activity

	S. aureus					*S. epidermidis*				
			% Nonsusceptible strains					% Nonsusceptible strains		
Test agent	MIC_{50}	MIC_{90}	*CLSI*	*EUCAST*	*% Bactericidal activity*	MIC_{50}	MIC_{90}	*CLSI*	*EUCAST*	*% Bactericidal activity*
Rifampin	0.015	4	11.11	16.67	0	0.015	0.015	5.26	5.26	27.78
Vancomycin	1	2	5.56	5.56	55.56	2	4	0	0	94.74
Tigecycline	0.25	0.5	—	0	0	0.12	0.25	—	0	5.26
Ciprofloxacin	>8	>8	66.67	66.67	66.67	1	>8	47.37	47.37	73.33
Cotrimoxazole	1	2	5.56	5.56	27.78	2	>32	42.10	42.10	6.25
Cloxacillin	>32	>32	66.67	66.67	50	0.5	>32	68.42	68.42	50
Clindamycin	0.12	>8	11.11	11.11	0	0.25	>8	31.58	36.84	7.69
Daptomycin	0.5	1	0	0	77.78	0.5	1	0	0	78.95
Fosfomycin	1	16	—	5.56	0	1	4	—	0	5.26

Vancomycin has traditionally been the first-line agent for PJI caused by multidrug-resistant staphylococci strains. However, owing to the recent increase of the MIC values that has been detected in some strains from both species, its use should be undertaken with caution.[30] In this sense, our study has registered one vancomycin nonsusceptible *S. aureus* strain (5.6%). On the other hand, the bactericidal activity of vancomycin was higher in *S. epidermidis* compared with that observed in *S. aureus* (94.74 vs 55.56%), potentially constituting an additional drawback for the use of this antibiotic.

Rifampin, another commonly used antibiotic with outstanding efficacy against these types of clinical isolates, showed high *in vitro* activity against both staphylococcal species in our series. However, this antibiotic worked as a bacteriostatic agent for most of the tested strains, thus making it necessary to be used in combination with other antimicrobials to avoid resistance development.[11,31] Tygecicline, daptomycin and fosfomycin could be interesting alternatives for antimicrobial therapy. Our results show that tygecicline inhibited all the tested strains, with no bacterial resistance detected. However, MBC data showed that this antibiotic worked as a bacteriostatic agent against most of the tested strains.

Daptomycin has become a viable i.v. alternative to vancomycin because of its high level of antimicrobial and antibiofilm activity, as previously reported.[10,32] According to our data, daptomycin not only inhibited all the tested strains but also showed high bactericidal activity for both bacterial species (77.78% for *S. aureus* and 78.95% for *S. epidermidis*). These results are in accordance with those of other studies.[33] However, the need for parenteral administration made daptomycin an alternative only for patients requiring i.v. therapy. Other oral alternatives are therefore necessary for treatment of patients outside the hospital setting.[16]

Finally, fosfomycin seemed to be an efficient agent, with only one *S. aureus* nonsusceptible strain found (5.56%), although this antibiotic exhibits a mostly bacteriostatic behavior.

Regarding the other tested antibiotics, antimicrobial activity findings generally resulted in differences between both staphylococcal species. Cotrimoxazole, as well as clindamycin, showed a better response against *S. aureus* than against *S. epidermidis*. In fact, all the MRSA strains tested in our study were cotrimoxazole-susceptible. This antibiotic has previously exhibited substantial *in vitro* activity against most MRSA strains.[12] Both antimicrobials could be administered orally, thus increasing their attractiveness for the management of some patients.

In conclusion, MRSA strains are an increasingly prevalent cause of PJIs; consequently, the use of more efficient antibiotics is needed. According to our data, rifampin, tigecycline, daptomycin and fosfomycin showed high *in vitro* activity against most of the tested staphyloccal strains isolated from the PJIs. *In vitro* results should be considered when determining the therapy for these patients, along with other pathogenic aspects such as biofilm development and prosthesis removal.

ACKNOWLEDGEMENTS

This work was supported by Comunidad de Madrid (BITI-CAM project S2009/MAT-1472) and Ministerio de Educación y Ciencia (project CONSOLIDER FUNCOAT CSD2008-00023). DMM was funded by the Fundación Conchita Rábago de Jiménez Díaz. AOP and GDP were funded by the Comunidad de Madrid. We acknowledge Mr Oliver Shaw for his help with English language.

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