

Infectious prosthetic hip joint loosening: bacterial species involved in its aetiology and their antibiotic resistance profiles against antibiotics recommended for the therapy of implant-associated infections

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SUMMARY

Reliable microbiological diagnosis along with surgery and prolonged antibiotic therapy are key elements in the management of prosthetic-joint infections (PJIs). The purpose of this study was to characterize antibiotic resistance profiles of bacteria involved in the aetiology of PJIs. A total of 33 bacterial isolates cultured from 31 patients undergoing exchange of total hip prostheses were analyzed. The diagnostic approach toward isolation of prosthesis-associated microorganisms included sonication of retrieved implants and conventional cultures of periprosthetic tissues and synovial fluid. The *in vitro* resistance profiles of bacterial isolates were determined in relation to antibiotics recommended for the therapy of PJIs using the disc diffusion method, E-tests[®] and broth microdilution system. Coagulase-negative staphylococci (CNS) were predominant microorganisms followed by *Staphylococcus aureus*, *Enterobacter cloacae*, *Streptococcus mitis*, and *Propionibacterium acnes*. Twenty out of 30 and 12 out of 30 staphylococcal isolates were methicillin- and multi-drug resistant, respectively. Only two isolates were rifampicin-resistant. All staphylococci were susceptible to glycopeptides and linezolid. This paper stresses the pathogenic role of staphylococci in patients suffering from implant loosening and reports high methicillin- and multidrug-resistance rates in these bacteria. Hence, antimicrobial susceptibility tests of individual bacterial isolates must always be performed to guide selection of the optimal therapeutic option.

KEY WORDS: Prosthetic-joint infection, Antibiotics, Resistance, Sonication, Staphylococci.

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INTRODUCTION

Although prosthetic joint implantations improve patients' quality of life, these procedures are burdened with a risk of severe complications, including aseptic loosening and prosthetic joint infection (PJI). Infectious implant failures have serious consequences for the patient, as they may lead to subsequent bone infection,

and also for society due to prolonged hospitalization, long and expensive treatments, and multiple surgeries (Esteban *et al.*, 2008; Cobo and Del Pozo, 2011).

Correctly distinguishing between biomaterial-related infections and aseptic loosening, which is critical for further therapeutic decisions, is of the utmost significance. Nevertheless, patients with delayed (those that develop 3 to 24 months after surgery) infections usually present with subtle signs and symptoms, such as implant loosening, persistent joint pain, or both, which can be difficult to distinguish from aseptic failure (Zimmerli and Trampuz, 2004). The ma-

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majority of patients suffering from late infections (more than 24 months after surgery) usually develop subacute infections following unrecognized bacteremia (Trampuz and Zimmerli, 2005; Zimmerli, 2006). In fact, oligosymptomatic PJIs cannot be diagnosed until microbiological evaluation is performed. The diagnostic problems are increased by the pathogenesis of PJIs associated with the production of microbial biofilm on the implant surface, intracellular survival of causative microorganisms and the presence of small-colony variants (SCVs) of bacteria (Nelson *et al.*, 2005, Esteban *et al.*, 2008, Monsen *et al.*, 2009).

Hence, reliable microbiological diagnosis, along with surgical procedures and prolonged appropriate antibiotic therapy are key elements in the management of PJIs (Senneville *et al.*, 2011). For successful antimicrobial therapy, in turn, identification of the causative microorganism and its susceptibility testing are pivotal (Holinka *et al.*, 2011; Senneville *et al.*, 2011).

The purpose of the study was to analyse the spectrum of bacterial species involved in the pathogenesis of prosthetic hip joint loosening in the aspect of the development of clinical manifestations of PJI, and to characterize antibiotic resistance profiles among the cultured isolates.

MATERIALS AND METHODS

Bacterial isolates characterized in the study were cultured from 31 patients undergoing one stage revision of a total hip prosthesis due to:

- implant loosening without accompanying clinical manifestations of infection (12 patients);
- implant failure accompanied by the development of a sinus tract communicating with the prosthesis indicative of an ongoing periprosthetic infection (19 patients).

The two groups of patients underwent the surgical procedures at the Orthopaedics and Traumatology Ward of the Clinical Hospital in Lublin, Poland and at the Public Clinical Hospital in Otwock, Poland, respectively.

The mean age of the 12 patients with the PJI symptom-free implant loosening was 68.1 ± 10.3 years (range between 50 and 81 years). The

mean time to the onset of the loosening symptoms in these patients was 66 ± 65.7 months (range between 8 to 169 months).

The mean age of 19 patients with presumed PJI was 68.1 ± 10.7 years (range between 39 and 84 years). The mean time to the onset of the loosening symptoms was 34.4 ± 34.7 months (range between 2 and 132 months).

The clinical data of the patients have been summarized in Table 1.

Sample collection

Intraoperatively, tissue samples from the close proximity of the implant and demonstrating the most obvious inflammatory changes were collected for microbiological studies. At least three tissue samples were collected from each patient. The synovial fluid was collected intraoperatively from patients with the clinical diagnosis of aseptic loosening for microbiological culture. The explanted prosthetic components were placed in 1-litre sterile jars. The specimens were processed by the microbiology laboratory within 2 hours.

Conventional microbiological methods

Synovial fluid was inoculated in 100 μ l aliquots onto a set of routine aerobic and anaerobic bacteriologic media. The plates were incubated at 35°C-37°C for up to 14 days.

Tissue specimens were inoculated into thioglycollate broth and incubated at 35°C-37°C. Cloudy thioglycollate broth was subcultured onto conventional bacteriologic media.

Sonication of removed prostheses

Five hundred milliliters of sterile saline were added to each container. The container was vortexed for 30 seconds and subsequently subjected to sonication (Branson[®] Ultrasonic Cleaner G.HEINEMANN) for 7 minutes at a temperature of 20°C. Sonication was followed by additional vortexing for 30 seconds (Trampuz *et al.*, 2007; Monsen *et al.*, 2009). The resulting sonicate fluid was removed under aseptic conditions and placed into 50-ml sterile Falcon tubes. Samples were then centrifuged at 4000 rpm for 20 minutes; 100 μ l aliquots of the sedimented sonicate fluid were inoculated onto a set of routine aerobic and anaerobic bacteriologic media. Incubation (at 35°C-37°C) lasted

TABLE 1 - Clinical data and results of the analysis of systemic and local levels of inflammatory markers among culture-positive patients included in the study.

Patient no.	Age/gender	Time to the onset of the loosening symptoms (months)*	No. of previous revisions	ESR (mm/h)	CRP (mg/l)	Synovial fluid leukocyte count and differential		Clinical manifestations of PJI	
						Leukocytes (cells/ μ l)	Neutrophils (%)		
4	62/F	13	2	88	6,3	12890	99	Absent	
26	75/F	8	1	49	28,09	1289	94		
18	74/F	20	0	57	31,53	NC	NC		
19	61/F	37	0	42	36,76	2606	94		
17	81/F	32	2	27	1,2	7069	97		
6	81/F	156	0	39	0,8	5130	72		
11	74/F	169	0	52	36,67	6503	95		
31	68/F	17	0	13	13,29	149600	95		
40	50/F	18	0	19	0,74	37600	97		
49	51/F	160	1	59	18,3	NC	NC		
56	70/F	129	1	91	72,99	54860	95		
57	71/F	33	0	52	11,89	7000	81		
O1.	61/M	12	0	50	11,9	ND	ND		Sinus tract
O2.	39/M	84	0	66	29,5				
O3.	59/M	21	0	50	1,6				
O4.	70/M	24	1	76	51,4				
O5.	65/M	36	0	46	19,5				
O6.	76/M	2	3	58	40,6				
O7.	60/M	32	0	64	29,8				
O8.	78/M	3	3	28	2,7				
O10.	75/M	9	0	46	6,8				
O11.	84/F	36	0	26	3,6				
O14.	65/F		0	60	11,7				
O15	58/M	28	0	65	35,8				
O16.	69/F	16	2	60	7,3				
O19.	71/F	96	1	74	93				
O20.	65/M	18	0	20	4,6				
O21.	78/F	36	3	76	68,5				
O22.	75/F	21	1	60	15,3				
O23.	84/M	132	0	22	0,8				
O24.	62/M	14	2	70	0,2				

*Time from implantation or previous revision; NC – material not collected; ND – not done ; dark background in the columns was used to highlight values above cut-off; red font was used to highlight patients with elevated ESR and/or CRP level accompanied by high synovial fluid leukocyte count and neutrophil percentage; F-female, M-male.

for up to 14 days. All media were inspected daily for microbial growth. The culture result was considered positive if there were at least 5 colony-forming units of the same organism on either plate. Isolated microorganisms were identified to the species level using commercially available biochemical tests (API, BioMérieux, France).

Antibiotic sensitivity testing

Antibiotic sensitivity testing was performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations. The applied methods included disc diffusion (Becton Dickinson discs), E-tests® (bioMérieux, France), and the broth microdilution system (ATB™ ANA, bioMérieux, France).

Definition of PJIs - how were culture results interpreted?

In the first group of 12 patients prosthesis failure occurred in the absence of clinical manifestations of PJI such as a sinus tract and/or purulence in the affected joint. The microbiological diagnostic proceedings were supplemented by the analysis of local (synovial fluid leukocyte count and differential) and systemic markers of inflammation (erythrocyte sedimentation rate/ESR, C-reactive protein/CRP) in order to verify the clinical significance of positive culture results and to exclude false-positive culture results.

The clinical diagnosis of PJI associated with implant loosening in the next group of 19 patients was made on the basis of the development of a sinus tract communicating with the prosthesis. This is a definitive symptom of an ongoing periprosthetic infection according to recommendations of Parvizi *et al.* (2011). The microbiological diagnostic proceedings were supplemented by the analysis of systemic markers of inflammation (ESR, CRP) in order to verify the infectious nature of the implant failure and to interpret the clinical significance of positive culture results.

According to the recommendations of Parvizi *et al.* (2011), a definite PJI exists when there is a sinus tract communicating with the prosthesis, or a pathogen is isolated by culture from 2 or more separate tissue or fluid samples obtained

from the affected joint, or when 4 of the following 6 criteria exist:

- a) Elevated ESR (>30 mm/h) or serum CRP (>10 mg/l) concentration (Trampuz *et al.* 2007; Parvizi *et al.* 2011)*;
- b) Elevated (>1700 cells/μl) synovial leukocyte count (cut-off value adopted from Trampuz *et al.* 2007)**;
- c) Elevated (>65%) synovial neutrophil percentage (cut off value adopted from Trampuz *et al.* 2007)**;
- d) Presence of purulence in the affected joint;
- e) Isolation of a microorganism in one culture of periprosthetic tissue or fluid.

Greater than five neutrophils per high-power field in five high-power fields observed from histologic analysis of periprosthetic tissue at x 400 magnification***

*the levels of systemic markers of inflammation were determined among all patients in the direct preoperative period;

**the synovial leukocyte and differential was assessed only among patients with a clinical diagnosis of aseptic loosening;

***pathohistological examination was not performed in the study. Hence, criteria a-e were taken into account for the final interpretation of culture results.

All the culture-positive patients included in this study fulfilled the above mentioned criteria. Table 1 demonstrates the levels of inflammatory mediators for individual patients in whom clinically significant results of microbiological culture were obtained.

RESULTS

Microbiological culture results from patients with implant loosening without accompanying clinical manifestations of infection; antimicrobial resistance profiles of cultured bacteria

Culture results and antibiotic resistance profiles of the individual bacterial isolates cultured from the patients are summarized in Table 2.

All (12) patients from the group were positive for bacterial growth in the sonicate fluid. Periprosthetic-tissue and synovial fluid cultures yielded bacterial growth among seven and five patients, respectively.

TABLE 2 - Culture results and antibiotic resistance profiles of the cultivated bacterial species.

No. of patient n=31	Type of specimen			Microorganism cultured	Resistance profile of the microorganism cultured
	S-F	SVF	PT (no.)		
4	+	-	+ (1)	<i>S. epidermidis</i>	MR, MLS _B , AMGL, RA*
6	+	-	-	<i>S. warneri</i>	MR
11	+	-	+ (2)	<i>E. cloacae</i>	G, AN
17	+	+	-	<i>S. epidermidis</i>	E, SXT, AMGL*
18	+	NC	+ (3)	<i>S. epidermidis</i>	MR, MLS _B , SXT, FA*
19	+	+	+ (4)	<i>S. epidermidis</i>	MR
26	+	+	-	<i>S. epidermidis</i>	MR, MLS _B
31	+	+	+ (3)	<i>S. aureus</i>	MR, MLS _B , CIP*
40	+	+	+ (1)	<i>S. epidermidis</i>	MR
				<i>P. acnes</i>	Full susceptibility to antibiotics tested
49	+	NC	-	<i>S. epidermidis</i>	AMGL
56	+	-	-	<i>S. mitis</i>	P, AMP
57	+	-	+ (2)	<i>S. epidermidis</i>	MR, SXT, AMGL*
O1	+	NC	+ (3)	<i>S. epidermidis</i>	MR, MLS _B , SXT, CIP, AMGL*
O2	+		+ (2)	<i>S. epidermidis</i>	Full sensitivity to antibiotics tested
O3	-		+ (1)	<i>S. epidermidis</i>	FA, NN, AN
O4	+		+ (2)	<i>S. aureus</i>	MR
O5	-		+ (2)	<i>S. epidermidis</i>	MR, MLS _B , SXT, NN, AN*
O6	+		+ (3)	<i>S. aureus</i>	Full sensitivity to antibiotics tested
O7	+	NC	+ (3)	<i>S. epidermidis</i>	MR, E
O8	+		+ (3)	<i>S. epidermidis</i>	MR, SXT, MLS _B , RA, CIP, AMGL*
O10	+		+ (2)	<i>S. simulans</i>	MR
				<i>S. capitis</i>	MR
O11	+		+ (1)	<i>S. epidermidis</i>	Full sensitivity to antibiotics tested
O14	-		+ (2)	<i>S. epidermidis</i>	MLSB
O15	+		+ (3)	<i>S. aureus</i>	Full sensitivity to antibiotics tested
O16	+		+ (3)	<i>S. warneri</i>	MR, MLS _B , AMGL*
O19	+		+ (3)	<i>S. epidermidis</i>	MR, AMGL, CIP*
O20	+		+ (2)	<i>S. lugdunensis</i>	Full sensitivity to antibiotics tested
O21	+		+ (3)	<i>S. epidermidis</i>	MR, SXT, AMGL*
O22	+		+ (1)	<i>S. epidermidis</i>	MR
O23	+		-	<i>S. cohnii</i>	MR, MLS _B , AMGL*
O24	NC		+ (2)	<i>S. aureus</i>	FA

NC – not collected; S-F – sonicate fluid; SVF – synovial fluid; PT – periprosthetic tissues (number of tissue samples from which growth of the same bacterial species was obtained); +/- : positive/negative culture result; MR- methicillin (tantamount to resistance to all beta-lactam drugs); MLSB –macrolides, linkosamides and group B streptogramins; AMGL – aminoglycosides; SXT – co-trimoxazole; CIP – ciprofloxacin; FA – fusidic acid; G – gentamicin; NN-tobramycin; AN - amikacin; E – erythromycin; RA – rifampicin; P- penicillin; AMP - ampicillin; * - multidrug resistance. no: 4-57: patients whose prosthesis failure occurred in the absence of clinical manifestations of PJI; no. O1-O24: patients with the clinical diagnosis of PJI based on the development of a sinus tract communicating with the prosthesis.

Staphylococci represented the overwhelming majority of microorganisms cultured from patients with a clinical diagnosis of aseptic implant loosening. Coagulase-negative staphylococci (CNS) were cultured from nine out of the 12 patients and they were represented by eight *Staphylococcus epidermidis* isolates and one *Staphylococcus warneri*.

In one patient (no. 40) the sonicate fluid culture revealed the growth of two microorganisms: *S. epidermidis* and *P. acnes*, whereas the synovial fluid and periprosthetic tissue cultures yielded only *S. epidermidis* in spite of prolonged incubation in anaerobic conditions. The remaining three patients were infected with *Staphylococcus aureus*, *Enterobacter cloacae*, and *Streptococcus mitis*.

Methicillin resistance was detected among eight out of ten staphylococcal isolates among which there was one *S. aureus* (MRSA, methicillin-resistant *S. aureus*) whereas the remaining isolates were represented by CNS.

In addition to methicillin resistance, staphylococcal isolates were found resistant to macrolides, lincosamides, and group B streptogramins (4 isolates), aminoglycosides (4 isolates), and co-trimoxazole (3 isolates). Rifampicin, ciprofloxacin and fusidic acid resistance was detected among three separate staphylococcal isolates. Five out of ten staphylococcal isolates were considered multidrug-resistant (MDR) as they demonstrated resistance to at least three groups of antimicrobial drugs. MDR isolates were methicillin-resistant with the exception of one isolate (no. 17).

Among other microorganisms cultured *E. cloacae* was found resistant to gentamicin and amikacin whereas *S. mitis* demonstrated *in vitro* resistance to penicillin and ampicillin.

Microbiological culture results from patients with prosthesis failure accompanied by the development of a sinus tract; antimicrobial resistance profiles of cultured bacteria

Culture results and the antibiotic resistance profiles of the individual bacterial isolates cultured from patients with a clinical diagnosis of PJIs are summarized in Table 2. Among the 19 patients, positive culture results from periprosthetic tissues were obtained among 18 patients

whereas the sonicate fluid cultures yielded microbial growth in 15 patients.

Staphylococci represented the only group of cultured microorganisms. *S. epidermidis* was the most frequently isolated species - it was cultured from 11 patients. Other members of CNS included *S. warneri* (2 isolates), *S. lugdunensis* (1 isolate), and single isolates of *S. simulans* and *S. captitis* which were cultured from one patient. *S. aureus* was cultured from four patients.

Methicillin resistance was detected among 12 out of 20 staphylococcal isolates among which there was one MRSA isolate whereas the remainder were represented by CNS. In addition to methicillin resistance, staphylococci demonstrated MLS_B resistance phenotype (6 isolates), resistance to aminoglycosides (5 isolates), ciprofloxacin (3 isolates), and co-trimoxazole (4 isolates). Resistance to fusidic acid was detected in two isolates. One isolate demonstrated resistance to rifampin. Seven out of 20 staphylococcal isolates were considered MDR. All MDR isolates were simultaneously methicillin-resistant. Five isolates (two *S. epidermidis* isolates, two *S. aureus* isolates and one *S. lugdunensis* isolate) were sensitive to all antibiotics tested.

DISCUSSION

The goal of treatment of PJIs is to cure the infection, prevent its recurrence, and ensure a pain-free, functional joint. Identification of the microorganisms involved and their antimicrobial susceptibilities is crucial to guide a long-term antimicrobial treatment which must be combined with an appropriate surgical procedure to achieve therapeutic success (Trampuz and Zimmerli, 2005; Del Pozo and Patel, 2009; Zimmerli, 2012).

According to the literature data the most common microorganisms associated with the etiology of PJIs are coagulase-negative staphylococci (CNS) (30-43%) and *Staphylococcus aureus* (12-23%), followed by mixed flora (10-11%), streptococci (9-10%), Gram-negative bacilli (3-6%), enterococci (3-7%), and anaerobes (2-4%) (Trampuz and Zimmerli 2005; Moran *et al.*, 2007; Sharma *et al.*, 2008; Samuel and Gould, 2010; Zimmerli and Moser, 2012; Mo-

lina-Manso *et al.*, 2013). Indeed, in our study, CNS with the predominant role of *S. epidermidis* represented the overwhelming majority of microorganisms involved in the pathogenesis of implant dysfunction. *S. epidermidis* was cultured from approximately 60% of patients irrespective of the clinical manifestation of implant dysfunction. *S. aureus*, in turn, was more frequently cultured from patients with a clinical diagnosis of presumed infection (21%) than from those in whom the prosthesis loosening occurred without clinical symptoms suggestive of PJI (8%). This observation is consistent with the significant pathogenic potential of *S. aureus* and a more aggressive course of infections caused by this species, usually manifested clinically. Other bacterial genera such as *E. cloacae*, *P. acnes* (in a mixed culture with *S. epidermidis* in the sonicate fluid) and *S. mitis* were cultured from single patients and, interestingly, only from those who did not develop clinical manifestations of PJI and were initially classified as suffering from aseptic loosening.

The pathogenesis of infections associated with biomaterials involves complex interactions between the causative microorganisms, the implant and the host. PJIs are primarily caused by microorganisms growing in biofilms - structures in which bacteria live clustered together in a highly hydrated extracellular matrix attached to a surface where they are protected from host immune responses and antimicrobials (Trampuz and Widmer, 2006; Trampuz and Zimmerli, 2006; Esposito and Leone, 2008; Samuel and Gould, 2010; Cobo and Del Pozo, 2011). Other strategies of microbial survival and persistence accounting for the pathogenesis of PJIs, and associated with therapeutic problems, include the presence of bacteria inside host cells such as osteoblasts and the small colony variants (SCVs), the last of which may be induced after treatment with gentamicin (Nelson *et al.*, 2005; Sendi *et al.*, 2006; Monsen *et al.*, 2009; Maduka-Ezeh *et al.*, 2012).

The above-mentioned aspects of the pathogenesis of PJIs must be taken into account to increase the chance of successful eradication of infection. The choice of antimicrobial agents applicable to the treatment of PJIs according to the pathogen and its antimicrobial susceptibility has been published and recommended

(Trampuz and Zimmerli, 2005; Trampuz and Widmer, 2006; Trampuz and Zimmerli, 2006; Zimmerli, 2006; Samuel and Gould, 2010; Zimmerli and Moser, 2012).

Optimal antimicrobial therapy has been best defined in PJIs of staphylococcal aetiology. Rifampin combination regimens have been reported to demonstrate excellent activity on susceptible adherent staphylococci existing in the stationary mode of growth. Their activity has been proven *in vitro*, in animal models, and in several clinical studies (Drancourt *et al.*, 1997; Trampuz and Zimmerli, 2005; Zimmerli, 2006; Aboltins *et al.*, 2007; Esposito and Leone, 2008; Senneville *et al.*, 2011; Cobo and Del Pozo, 2011; Zimmerli and Moser, 2012; Molina-Manso *et al.*, 2013). The results of our study are also suggestive of a potential therapeutic usefulness of rifampicin, as resistance to this drug was detected *in vitro* in only two isolates (*S. epidermidis*). A similar trend was reported by Moran *et al.* (2007) who found that only five out of 74 CNS strains and none among *S. aureus* strains were resistant to rifampin. However, in our study, this resistance phenotype was additionally accompanied by resistance to multiple antimicrobial agents which has drastically narrowed down the spectrum of available therapeutic options.

Rifampin should always be combined with another drug to prevent the emergence of resistance. Quinolones are considered excellent combination drugs due to their good bioavailability, activity, and safety (Zimmerli, 2006). Ciprofloxacin resistance rates among staphylococci isolated from patients enrolled in our study did not exceed 15%. Nevertheless, resistance to this drug was additionally accompanied by multi-drug resistance in all isolates. According to the available literature, resistance of staphylococci to quinolones is considered an increasing problem and they should not be used in the therapy of infections caused by MRSA due to a risk of emergence of resistance (Drancourt *et al.*, 1997; Stein *et al.*, 1998; Zimmerli, 2006; Aboltins *et al.*, 2007).

Other anti-staphylococcal drugs which have been combined with rifampin include co-trimoxazole, fusidic acid or linezolid (Zimmerli, 2006; Aboltins, 2007; Cobo *et al.*, 2013).

Linezolid, to which all staphylococcal isolates

cultured in our study were found sensitive, has been reported to be an attractive agent for bone and joint infections owing to its excellent oral absorption, its bone tissue penetration, activity against MDR Gram-positive bacteria including all CNS species and the good results in experimental models when combined with rifampin (Cobo *et al.*, 2013). Cobo *et al.* (2013) reported satisfactory results of chronic PJI treatment with linezolid. Among patients who completed treatment, 19 (86%) demonstrated clinical and microbiological cure. Twenty-two (88%) patients tolerated the drug without major adverse effects, although a global decrease in the platelet count was observed. These authors observed that side-effects associated with the antibiotic administration such as anaemia and thrombocytopenia were reversible after withdrawal of linezolid and suggested that its toxicity may be reduced if the drug is combined with rifampin due to the decrease in serum levels of linezolid or if shorter courses of antibiotics are considered.

According to Aboltins *et al.* (2007), rifampicin and fusidic acid have good activity against most staphylococci and have retained activity against the majority of methicillin-resistant and fluoroquinolone-resistant staphylococci. In our study, the overall resistance rate against fusidic acid was low and only one out of three fusidic acid resistant isolates was methicillin-resistant. Aboltins *et al.* (2007) highlighted the fact that these two antimicrobial agents are well-absorbed after oral administration, and demonstrate excellent penetration into tissues and the intracellular space. Drancourt *et al.* (1997) mentioned that *in vitro* studies have shown that the combination of fusidic acid with rifampicin prevents the selection of staphylococci resistant to either drug.

It should be emphasized that methicillin resistance was a predominant resistance phenotype among staphylococci cultured from patients enrolled in the study as it was detected among 20 out of 30 isolates. As Esposito and Leone (2008) mentioned, both *S. aureus* and the CNS increasingly show resistance to methicillin. In the study reported here, methicillin resistance rates observed for *S. aureus* were much lower than those observed for the CNS. Namely, MRSA were isolated from single culture-posi-

tive patients irrespective of the clinical classification of the implant loosening. Similarly, in the study conducted by Moran *et al.* (2007), 8% of patients with PJIs grew MRSA whereas 60% of CNS were resistant to methicillin. In the study conducted by Sharma *et al.* (2008) only 35% of CNS isolates cultured from cases of infected arthroplasty were methicillin-sensitive. In our study, methicillin resistance rates among CNS oscillated in the region of 70% regardless of the clinical presentation of the prosthesis failure.

Our observations indicate that methicillin resistance, detected particularly frequently among CNS, is a common trend giving rise to concerns, especially as this resistance phenotype is frequently associated with additional resistance to other antimicrobial agents. MDR isolates accounted for 40% (12 out of 30 isolates) of staphylococci cultured from patients enrolled in this study. The overwhelming majority of the MDR staphylococci were represented by CNS; there was only one MDR *S. aureus* isolate. The MDR phenotype was simultaneously associated with resistance to methicillin among all but one isolate.

Multidrug resistance is a disturbing trend adversely influencing the treatment of PJIs further complicated by the adherence of pathogenic bacteria to the implant surface and the formation of biofilm. Stein *et al.* (1998) suggested long-term oral ambulatory treatment with high doses of co-trimoxazole as an effective alternative to the conventional medico-surgical treatment of chronic MDR (susceptible only to glycopeptides and co-trimoxazole) *Staphylococcus*-infected orthopaedic implants. However, this treatment regimen could not be administered to eradicate the majority of the MDR staphylococci isolated in our study since as many as seven of the 12 MDR isolates were resistant to co-trimoxazole.

Taking into account the increasing demand for arthroplasty due to an aging population on the one hand, and the growing problem of antimicrobial resistance complicated by periprosthetic microbial adhesion, the ability of pathogens to invade and survive within host cells and to select for SCVs on the other, one may expect that PJIs will continue to present a challenge in terms of their diagnosis and treatment.

This paper stresses the pathogenic role of staphylococci irrespective of the clinical presentation of implant loosening (aseptic vs. septic) and reports high rates of methicillin- and multidrug-resistant bacteria belonging to this genus with particular emphasis on CNS. These observations, in turn, highlight the significance of antimicrobial susceptibility studies of individual bacterial isolates before selection of the optimal antibiotic treatment. Moreover, it should be remembered that the choice of an appropriate antimicrobial agent must depend not only on the susceptibility profiles of the causative microorganisms determined *in vitro* but it also on its potential effectiveness against pathogens existing in the form of biofilms or within host cells.

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