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Antibiotic sensitivities of coagulase-negative staphylococci and *Staphylococcus aureus* in hip and knee periprosthetic joint infections: does this differ if patients meet the International Consensus Meeting Criteria?

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Introduction: Coagulase-negative staphylococci (CoNS) are the main pathogens responsible for prosthetic joint infections (PJIs). As normal inhabitants of human skin, it is often difficult to define if they are contaminants, or if they have an active role in initiating infection. This study aims to evaluate differences in CoNS organisms (*Staphylococcus hominis, Staphylococcus capitis*, *Staphylococcus haemolyticus*, *Staphylococcus warneri)* and *Staphylococcus aureus* in terms of isolation rate and antimicrobial susceptibility from patients who met the International Consensus Meeting (ICM) criteria for PJIs and those who did not.

Methods: Staphylococci isolates from January 2014 to December 2015 retrieved from patients undergoing revision joint arthroplasty were classified in accordance with criteria established by the ICM of Philadelphia.

Results: As per the consensus classification, 50 CoNS and 39 *S. aureus* infections were recognized as pathogens, while 16 CoNS and four *S. aureus* were considered as contaminants. Frequency of isolation of *S. aureus* was significantly higher in infected patients than in those without infection, while no significant differences were observed among CoNS. Resistance to levofloxacin, erythromycin, gentamicin trimethoprim/sulfamethoxazole, and rifampicin was significantly more frequent in *S. haemolyticus* than in the other species, as well as resistance to erythromycin and gentamicin in *S. hominis*. In comparison to *S. aureus,* CoNS were significantly more resistant to daptomycin and gentamicin and more susceptible to rifampicin.

Conclusion: CoNS, other than *Staphylococcus epidermidis,* are frequently isolated from PJIs, and their infective role and antimicrobial susceptibility need to be assessed on an individual patient basis. *S. haemolyticus* seems to emerge as responsible for PJI in a large volume of patients, and its role needs to be further investigated, also considering its pattern of resistance.

Keywords: periprosthetic joint infections, antibiotics susceptibility, *Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus capitis, Staphylococcus warneri*, *Staphylococcus aureus*

Introduction

Management of established prosthetic joint infection (PJI) requires a combination of pathogen-specific antibiotics and surgical intervention. The choice of antibiotic is dependent upon the microbiological cultures of specimens obtained preoperatively or intraoperatively, with a greater sensitivity and specificity seen associated with tissue samples, compared to aspirations or swabs.[1,2]

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Due to the lack of laboratory assays with high sensitivity and specificity for PJI diagnosis, a multidisciplinary approach that combines results from microbiological, biochemical, and histological analyses, with clinical and radiological examination should be adopted. Therefore, several criteria for diagnosing PJI have been proposed by various scientific and medical societies.[3–6] Despite some differences, they all highlight the role of tissue culture and the need to discriminate between pathogens and contaminants, enabling antimicrobial susceptibility testing to target the causative agent, thus improving patient outcome.

Staphylococcus aureus and *Staphylococcus epidermidis* are the predominant pathogens responsible for PJI.[9,10] They are part of the natural skin microbiota, but can lead to devastating consequences should they establish themselves on the surface of an implant, produce biofilm, and evade host-immune defense.[11]

Improvement of methods for microbial identification has led to an increased rate of isolation of microorganisms, particularly coagulase-negative staphylococci (CoNS) and propionibacteria, which had been considered as mere contaminants in the past. The real meaning of these microorganisms in the pathogenesis of human diseases has still to be clarified, and CoNS are not always identified at species level by many clinical microbiology laboratories. However, as the pathogenic significance of CoNS increases, it may become important to learn more about the epidemiology and pathogenic potential of individual species. Their pathogenicity includes adhesion to human epithelial cells, production of biofilms, and invasion and lysis of human cells.[12]

Of the CoNS species, evidence is growing on the role *Staphylococcus lugdunensis* has as a PJI pathogen.[13–15] Other CoNS, such as *Staphylococcus warneri, Staphylococcus haemolyticus, Staphylococcus capitis,* and *Staphylococcus hominis*, have been less frequently isolated and, consequently, less studied as causative agents of PJI, but can still have a role.[16] These organisms are often thought to be skin contaminants and have been shown to contaminate up to 32% of prosthetic joints during surgery.[17]

The aim of this study was to document the presence of *S. warneri, S. haemolyticus, S. capitis,* and *S. hominis* in patients with hip and knee PJI, and compare this to *S. aureus,* specifically to determine if a difference is seen in terms of isolation rate and antimicrobial susceptibility, between strains isolated from patients who met the criteria for PJI diagnosis, compared to those who did not.

Methods

Patient characteristics

All patients presenting to our institute, a 300-bed reference center specializing in orthopedic surgery sited in Northern Italy, and undergoing revision surgery for suspected hip or knee PJI between January 2014 to December 2015 were considered eligible for the study. Since our hospital is one of the leading hospitals for prosthetic joint surgery at national level, the population served includes patients from all over Italy.

All patients signed an informed consent to participate in the study which had previously received the approval of the Galeazzi Institute Review Board (composed of ten members including medical doctors and basic scientists).

For each patient, erythrocyte sedimentation rate (ESR, mm/hr, laboratory range <20 mm/hr for females, <24 mm/hr for males) and C-reactive protein (CRP: laboratory range 0–5 mg/L) were measured on hospital admission.

Diagnosis of infections was based on criteria established by the International Consensus Meeting (ICM) held in Philadelphia in 2013.[5,6] We have chosen to use these criteria to define the presence of a PJI as this takes into account previously defined criteria,[3,4] and is the definition routinely used at our institute. The ICM criteria are dependent upon a combination of clinical examination, hematological, and microbiological results. However, despite the accuracy of these criteria, patients who did not meet the entire criterion were treated as such if we had a high index of suspicion of infection. Therefore, for the purposes of this study, we retrospectively divided our patient cohort by those meeting the criteria (Group A) and those who did not (Group B).

Sample collection

Four to six samples, or more, from the periprosthetic tissue as well as the implant (if removed) were collected from each patient of both groups during surgery prior to starting systemic antibiotics, unless the patient was systemically compromised. Postoperatively, for antibiotic prophylaxis, cefazolin (a first-generation cephalosporin antibiotic) was continued for 7 days or less, until culture results were known.

Samples were treated with dithiothreitol (DTT) in a closed-loop system for collection, transport, and treatment of samples within 1 hr from their arrival at the laboratory.[17,18] DTT has been shown to chemically detach bacteria from biofilm formed on the prosthetic implants and periprosthetic tissues, significantly increasing the rates of pathogen isolation.[2,7,8]

Samples were then plated on agar plates and inoculated in broth for aerobic and anaerobic cultures. Chocolate agar and MacConkey agar were incubated for 24 hrs in presence and absence of 10% CO_2, respectively. Mannitol salt agar and Sabouraud agar were incubated aerobically for 48 hrs. Finally, broths (Brain Heart Infusion and thioglycollate) were maintained for 15 days with daily visual inspection for bacterial growth; in case of turbidity and at the end of incubation, an aliquot (10 µL) of broths was plated on blood agar and Schaedler agar (only thioglycollate). Microbial identification and antibiotic susceptibility tests were carried out on a VITEK®2 Compact (bioMérieux, Marcy L'Etoile, France). Biochemical identification was confirmed by pyrosequencing analysis carried out on a PSQ96RA (Diatech, Jesi, Italy), as previously reported.[19] Antibiotic panel for staphylococci (Card AST 632, bioMerieux) included 14 antibiotics: fusidic acid, clindamycin, daptomycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, rifampicin, teicoplanin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole, and vancomycin, yielding MIC values and classifying the effectiveness as susceptible, intermediate, and resistant.[20]

Statistical analysis

Statistical analysis was performed using two-tailed Fisher's exact test when analyzing two groups directly, and Chi-squared test with 3 or 4 degrees of freedom to compare all four pathogen groups. The antibiotic sensitivities were grouped as sensitive or not (including intermediate and resistant). In addition, Student's *t*-test was used to compare bivariate data of means. A *p*-value of <0.05 was deemed to be statistically significant.

Results

Over the 24-month period, we obtained tissue samples from 580 patients. *S. haemolyticus, S. capitis, S. hominis,* and *S. warneri* were isolated from 66 patients (11.4%), and included 32 (48.5%) males, and 34 (51.5%) females (Table 1). *S. aureus* was isolated from 43 patients (7.4%), including 20 (46.5%) males and 23 (53.5%) females. The mean age of the cohort was 66.7 years (SD 13.7 years) (Table 2). No patient died in the study period and during the follow-up. Pyrosequencing confirmed biochemical identification at species level of all CoNS performed by Vitek system. In four patients, *S. aureus* was isolated with *S. epidermidis*, and in one with *Proteus mirabilis* and *Enterococcus faecalis*. Furthermore, *S. hominis* was isolated with *S. epidermidis* in one patient, and with *Propionibacterium acnes* in another. *S. capitis* was also isolated with *P. acnes* once.

Table 1 Pathogens isolated in each patient cohort

	Group A		Group B	
	n	%	n	%
Staphylococcus capitis	19	21.3	6	30
Staphylococcus hominis	14	15.7	6	30
Staphylococcus warneri	11	12.4	3	15
Staphylococcus haemolyticus	6	6.7	1	5.0
Staphylococcus aureus	39	43.8	4	20
Total	89	100	20	100

Patient cohorts

Patients fulfilling criteria for PJI diagnosis (Group A)

Fifty patients with CoNS pathogens fulfilled the ICM criteria for PJI diagnosis (74.2%): 27 females (54%) and 23 males (46.0%), with a mean age of 65.5 years. Hip and knee PJI was diagnosed in 26 and 24 patients, respectively. Their mean preoperative ESR and CRP values were 34.5 mm/hr and 18.8 mg/L. The most common CoNS species identified was *S. capitis* (Table 1). No differences were observed in isolation rate of CoNS between Group A and Group B (Table 1).

Thirty-nine patients with *S. aureus* also fulfilled the ICM criteria (90.7%): 20 females (51.3%) and 19 males (48.7%), with a mean age of 65.7 years (SD 13.9 years, range: 30–85 years). Twenty-four (61.5%) had an infected hip and 15 (38.5%) a knee PJI. Mean ESR was 58.6 mm/hr (SD 25 mm/hr) and mean CRP was 52.4 mg/L (SD 51.5 mg/L). The mean ESR was statistically significantly higher for patients with *S. aureus* (p=0.002) than for patients with CoNS as well as number of patients with ESR >30 mm/hr (p=0.016) and CRP >10 mg/L (p<0.001) (Table 2). Frequency of isolation of *S. aureus* from Group A was significantly higher than from Group B (p=0.04; Table 1).

Although follow-up was limited to only 2 years, two patients experienced a recurrence of the infection (one with *S. capitis* and one with *S. hominis*), while another was re-admitted to hospital for infection of the revised prosthesis due to *S. aureus* 33 months after prosthesis revision. In three patients with *S. aureus* infections, the same microorganism was isolated later from the spacer during second stage revision surgery, while another was re-admitted to hospital for wound infection due to *Klebsiella pneumoniae* and *Acinetobacter baumannii,* both resistant to carbapenems and carbapenemase producers acquired during rehabilitation in another hospital.

Patients not fulfilling ICM criteria (Group B)

S. warneri, *S. hominis*, *S. capitis,* and *S. haemolyticus* were isolated from 16 patients (25.8%) who did not fulfill the ICM

Table 2 Characteristics of patients of Group A and Group B

Characteristics		Group A		Group B		*p*-values		
		Staphylococcus aureus (n=39)	CoNS (n=50)	*S. aureus* (n=4)	CoNS (n=16)	*S. aureus* vs CoNS		Group A vs Group B
		n	n	n	n	Group A	Group B	CoNS
Gender	Male	20	23	1	9	0.150	0.248	0.176
	Female	19	27	3	7			
Age (years)	Mean (SD)	65.7 (13.9)	65.0 (12.3)	55.5 (17.8)	61.3 (16.3)	0.810	0.557	0.327
	Range	30–85	29–84	31–71	19–87			
Joint	Hip	24	26	3	9	0.126	0.331	0.221
	Knee	15	23	1	8			
ESR (mm/hr)	Mean (SD)	58.6 (25)	34.5 (24.5)	24.5 (/17.8)	24.5 (17.8)	0.002	0.266	0.209
	>30 mm/hr	28	20	0	4	0.016	0.484	0.202
	Range	5–100	7–99	3–26	5–55			
CRP (mg/L)	Mean (SD)	52.4 (51.5)	28.3 (3.65)	2.18 (1.45)	3.07 (2.7)	0.087	0.536	0.174
	>10 mg/L	34	26	0	0	<0.001	1.000	<0.011
	Range	3.2–255	0.5–485	0.3–3.5	0.8–9.9			

Abbreviations: CoNS, coagulase-negative staphylococci; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

criteria for PJI: seven females and nine males, with a mean age of 61.19 years. Nine hip and seven knee PJI were included.

Preoperative mean ESR and CRP were 24.5 mm/hr and 3.07 mg/L. *S. capitis* and *S. hominis* were the most common CoNS isolated from these patients (Table 1).

S. aureus was isolated from only four patients not fulfilling the ICM criteria (Table 1): three females and one male with a mean age of 55.5 years (SD 17.8 years). One knee and three hip implants were affected. The mean ESR was 12.7 (SD 11.9 mm/hr) and CRP was 2.18 (SD 1.45 mg/L). No statistically significant differences were seen comparing patients with *S. aureus* to patients with CoNS in the Group B cohort (Table 2).

None of the patients of this group reported complications in the follow-up after revision surgery. All the strains isolated from this group were classified as contaminants.

CoNS patient cohorts in Group A and Group B

No statistically significant difference was found between Group A and Group B CoNS patient cohorts in terms of their age, gender, and joint involvement. CRP was significantly higher in patients of Group A compared to Group B (p=0.011), while no differences between groups were observed for ESR (Table 2).

Antibiotic susceptibility

Antimicrobial susceptibility testing was performed for all isolates of both groups.

Patients fulfilling ICM criteria (Group A)

Table 3 demonstrates the antibiotic susceptibilities of the isolates of patients fulfilling the ICM criteria. The analysis of all the pathogens combined demonstrated a statistically significant difference among CoNS species. Resistance to levofloxacin, erythromycin, gentamicin, trimethoprim/sulfamethoxazole, and rifampicin was significantly more frequent in *S. haemolyticus* than in the other species (p<0.01 for levofloxacin, erythromycin, gentamicin, rifampicin, and p=0.03 for trimethoprim/sulfamethoxazole), as well as resistance to erythromycin and gentamicin in *S. hominis* (p<0.001). Methicillin resistance did not significantly differ among the tested CoNS, even though it was more frequently observed in *S. haemolyticus* (p=0.055). Frequency of multidrug-resistant (MDR) isolates, defined as resistance to at least three classes of antimicrobials significantly varied among CoNS (p=0.003), being more frequent in *S. hominis* (6/14 isolates) and *S. haemolyticus* (3/6 isolates) than in *S. capitis* (1/19 isolates) and *S. warneri* (0/11).

In comparison to *S. aureus,* CoNS were significantly more resistant to daptomycin and gentamicin (p=0.012) and more susceptible to rifampicin (p=0.03), while no significant differences were observed in methicillin resistance (p=0.144) and in isolation rate of MDR staphylococci (10/50 CoNS vs 15/39 *S. aureus*, p=0.062).

Patients not fulfilling ICM criteria (Group B)

Isolates of patients not meeting the ICM criteria for PJI (Group B) are reported in Table 4. Among all the pathogens, the only significant differences between the antibiotic sensitivities were the rate of resistance of *S. aureus* against clindamycin (p=0.017) and that of *S. capitis* to erythromycin (p=0.031). When comparing the statistical differences between *S. aureus* to the grouped CoNS, *S. aureus* was

Table 3 Susceptibility of staphylococci isolated from patients fulfilling PJI criteria (group A)

Antibiotic	*Staphylococcus capitis*			*Staphylococcus hominis*			*Staphylococcus warneri*			*Staphylococcus haemolyticus*			*Staphylococcus aureus*		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Fusidic acid	16	0	3	10	0	4	11	0	0	4	0	2	37	0	2
Clindamycin	16	0	3	7	0	7	11	0	0	3	0	3	29	0	10
Daptomycin	15	0	4	14	0	0	10	0	1	6	0	0	39	0	0
Erythromycin	14	0	5	2	0	12	9	0	2	0	0	6	24	1	14
Gentamicin	18	0	1	5	0	9	8	0	3	1	0	5	33	0	6
Levofloxacin	17	0	2	10	0	4	11	0	0	1	0	5	23	0	16
Linezolid	19	0	0	14	0	0	11	0	0	6	0	0	39	0	0
Oxacillin	11	0	8	6	0	8	9	0	2	1	0	5	27	0	12
Rifampicin	19	0	0	14	0	0	10	0	1	2	0	4	27	1	11
Teicoplanin	19	0	0	14	0	0	11	0	0	6	0	0	38	0	1
Tetracycline	18	0	0	6	2	6	8	1	2	1	2	3	29	1	9
Tigecycline	19	0	0	14	0	0	11	0	0	6	0	0	39	0	0
Trimethoprim/sulfamethoxazole	18	0	1	12	2	0	11	0	0	3	0	3	37	0	2
Vancomycin	19	0	0	14	0	0	11	0	0	6	0	0	39	0	0

Abbreviations: PJI, prosthetic joint infection; S, susceptible; I, intermediate; R, resistant.

Table 4 Susceptibility of staphylococci isolated from patients not fulfilling PJI criteria (group B)

Antibiotic	*Staphylococcus capitis*			*Staphylococcus hominis*			*Staphylococcus warneri*			*Staphylococcus haemolyticus*			*Staphylococcus aureus*		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Fusidic acid	5	0	1	4	0	2	3	0	0	0	0	1	3	0	1
Clindamycin	6	0	0	5	0	1	3	0	0	1	0	0	1	0	3
Daptomycin	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0
Erythromycin	6	0	0	4	0	2	2	0	1	0	0	1	1	0	3
Gentamicin	6	0	0	4	0	2	2	0	1	1	0	0	3	0	1
Levofloxacin	6	0	0	6	0	0	3	0	0	1	0	0	2	0	2
Linezolid	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0
Oxacillin	5	0	1	5	0	1	3	0	0	0	0	1	2	0	2
Rifampicin	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0
Teicoplanin	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0
Tetracycline	4	2	0	4	2	0	2	1	0	0	0	1	3	0	1
Tigecycline	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0
Trimethoprim/sulfamethoxazole	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0
Vancomycin	6	0	0	6	0	0	3	0	0	1	0	0	4	0	0

Abbreviations: PJI, prosthetic joint infection; S, susceptible; I, intermediate; R, resistant.

relatively more resistant to clindamycin (p=0.002) and erythromycin (p<0.001) than CoNS. Frequency of MDR isolates was significantly different among CoNS (p=0.003), reflecting what was observed for CoNS isolated from patients fulfilling ICM criteria. All *S. hominis* and *S. haemolyticus* isolates, but only one out of six *S. capitis* and 0 of three *S. warneri* were classified as MDR. Of the four *S. aureus* isolated in these patients, two were MDR. No differences were seen when MDR isolation rate of grouped CoNS was compared to that of *S. aureus* (p=1).

CoNS patient cohorts in Group A and Group B

Comparing the sensitivities of the CoNS isolated from patients fulfilling and not fulfilling the ICM criteria (Table 5), the only statistically significant difference between the two groups was levofloxacin susceptibility (p=0.009). Interestingly, methicillin resistance in Group B was about half of that observed in Group A. Although notable, this difference failed to reach statistical significance (p=0.052). In contrast, MDR isolates were more frequently seen in CoNS of group B than in Group A (p=0.027).

Table 5 Comparison of CoNS in Group A and B

Antibiotic	Group A				Group B				*p*- value
	Susceptible		Not susceptible		Susceptible		Not susceptible		
	N	%	N	%	N	%	N	%	
Fusidic acid	41	82	9	18	12	75	4	25	0.540
Clindamycin	37	74	13	26	15	94	1	6	0.093
Daptomycin	45	90	5	10	16	100	0	0	0.086
Erythromycin	25	50	25	50	12	75	4	25	0.079
Gentamicin	32	64	18	36	13	81	3	19	0.197
Levofloxacin	39	78	11	22	16	100	0	0	0.009
Linezolid	50	100	0	0	16	100	0	0	1.000
Oxacillin	27	54	23	46	13	81	3	19	0.052
Rifampicin	45	90	5	10	16	100	0	0	0.086
Teicoplanin	50	100	0	0	16	100	0	0	1.000
Tetracycline	33	66	17	34	10	63	6	38	0.798
Tigecycline	50	100	0	0	16	100	0	0	1.000
Trimethoprim/ sulfamethoxazole	44	88	6	12	16	100	0	0	0.059
Vancomycin	50	100	0	0	16	100	0	0	1.000

Abbreviation: CoNS, coagulase negative staphylococci.

Discussion

Staphylococci are recognized as the most frequent cause of PJI,[4] with a prevalence of *S. epidermidis* and other CoNS over *S. aureus*. Unfortunately, in the reported literature, CoNS are not identified at species level but are often clustered together and reported as CoNS, apart from *S. epidermidis* and, more recently, *S. lugdunensis*. To our knowledge, minimal data have been published on the sensitivity of these individual CoNS and on comparisons between the antimicrobial susceptibility rates of CoNS and *S. aureus* isolated from bone and joint infections.[21–25]

We have documented the current prevalence of these organisms in our patient cohort of 580 patients over a 2-year period (January 2014 to December 2015). This is a large patient cohort as our institute is a tertiary center for bone and joint infection, covering a large geographical area in Italy. The main pathogen isolated was *S. aureus*, but considered together, *S. warneri*, *S. capitis, S. hominis,* and *S. haemolyticus* were found in a larger proportion of patients. No acute hematogenous infections were observed among our CoNS patients, thus suggesting that the pathogen may infect the implant perioperatively, as recently proposed by other authors regarding *S. capitis*.[22] Indeed, CoNS are known to be less aggressive and therefore patients may present low-grade infections and some of the ICM criteria may not be met routinely, despite the presence of PJI.[12] Some differences in antimicrobial resistance were observed among CoNS, i.e., MDR isolates were more frequent in *S. hominis* and *S. haemolyticus* than in *S. capitis* and *S. warneri*. Interestingly, although *p*-value did not reach statistical significance, the rate of methicillin resistance in *S. haemolyticus* was higher than that observed for *S. hominis*, *S. warneri,* and *S. capitis*, even though the low number of isolates does not allow for any conclusion to be drawn.

Methicillin resistance has been shown to be more common among *S. epidermidis* and *S. haemolyticus* isolates than among *S. aureus*.[26,27] Moreover, they may act as a reservoir of staphylococcal cassette chromosome (SCC) elements for *S. aureus*, including SCC harboring the MR gene *mec* (SCC-*mec*) and the SCC-like arginine catabolic mobile element,[28,29] which favors staphylococcal colonization of the skin.

Resistance to rifampicin was more frequently observed in *S. aureus* than in CoNS. Rifampicin is considered one of the antibiotics of choice for treatment of bone and joint infections, due to its ability to penetrate staphylococcal biofilms and to maintain its activity during chronic and active stage of infection.[30,31] However, its use has been associated with the rapid emergence of resistant mutants, and therefore it is usually administered in combination with other agents.[31] In this study, the observed resistance rate to rifampicin in *S. aureus* does not greatly differ from that reported for other Italian isolates,[32] but is higher than other studies, despite similar susceptibility of CoNS.[23–25] In contrast, none of the staphylococci isolated from patients not fulfilling criteria for PJI was resistant to rifampicin. The high rate of resistance to rifampicin deserves further studies and some concerns arise regarding its use for treatment of PJIs, also considering that resistance to combination of rifampicin with other antibiotics, such as fluoroquinolones, is rising.[24]

Similarly, resistance to levofloxacin was more frequent in *S. haemolyticus* and *S. aureus* than in the comparators, and was also observed in *S. aureus* isolated from patients not fulfilling PJI criteria, but not in CoNS isolated from the same group of patients. Since 18 of 25 levofloxacin-resistant staphylococci were also methicillin resistant, it may be hypothesized that resistance to this fluoroquinolone could be associated with methicillin resistance, as suggested by others.[33,34] Considering that in Italy fluoroquinolones are widely used, it may be hypothesized that their use could have driven methicillin resistance development. However, because we have no data on previous antimicrobial therapy, this hypothesis cannot be confirmed.

On the whole, these results highlight the importance of choosing the correct drug prophylaxis taking into consideration the expected pattern of isolates following hip and knee PJI. Use of cefazolin in prophylaxis might be questioned considering the rate of methicillin resistance we observed. The common dominators with 100% sensitivity to all organisms were linezolid, tigecycline, and vancomycin.

However, vancomycin given prophylactically might increase the risk of infection due to the development of vancomycin resistant mutants, and Hawn et al. recommended combining it with a second agent, such as levofloxacin, to further reduce this risk.[35,36] On the other hand, linezolid and tigecycline are considered second-line antimicrobials and their use in prophylaxis is not advisable. A new regimen should be evaluated by considering local epidemiology and ability of antibiotics to "select" resistance mutants, and other factors not associated with antimicrobial resistance patterns but with pharmacokinetic and pharmacodynamic properties and with patients' characteristics.

Limitations of this study include that the results cannot be generalized as they are specific to our patient cohort. The results of this study do not reflect the surgical interventions undertaken by the responsible operating surgeon, as many factors should be taken into consideration in addition to the microbiological results, such as patient co-morbidities and soft tissue quality. A potential limit of this study could be the methods used to identify CoNS, as Vitek2 has shown some limitations in correctly identifying CoNS at species level compared to other more novel methods such as mass spectrometry.[37,38] Therefore, we decided to confirm biochemical identification of CoNS with pyrosequencing analysis that confirmed results obtained with Vitek2 for all CoNS isolates.

Conclusion

CoNS other than *S. epidermidis* are frequently isolated from PJIs, but their role in the pathogenesis of the infection needs to be assessed for each patient. Our data have shown some differences in the antimicrobial susceptibility of the species of CoNS, highlighting the importance of species identification. Moreover, some differences in antimicrobial susceptibility exist between isolates from patients fulfilling PJI criteria and those not, although this cannot be considered a valid criterion to discriminate pathogens from contaminants. Finally, *S. haemolyticus* seems to emerge as a responsible pathogen of PJIs, and its role needs to be further elucidated, also considering its pattern of resistance.

Disclosure

The authors report no conflicts of interest in this work.

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