Original Article

A Clinico-Microbiological Study of Prosthetic Joint Infections in an Indian Tertiary Care Hospital: Role of Universal 16S rRNA Gene Polymerase Chain Reaction and Sequencing in Diagnosis

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Abstract

Background: We determined the magnitude and clinico-microbiological profile of prosthetic joint infection (PJI) at a tertiary hospital. The diagnostic potential of 16S rRNA gene polymerase chain reaction (PCR) and sequencing on periprosthetic tissue samples was evaluated for the diagnosis of PJI. **Materials and Methods:** This ambispective cohort study consisted of patients who underwent primary or revision hip or knee arthroplasty from June 2013 to June 2017. The patients were classified as either infected or noninfected according to criteria set out by the musculoskeletal infection society (MSIS). Three to five periprosthetic tissue samples were collected from each patient for culture and 16S rRNA gene PCR sequencing. **Results:** Hundred and six patients were diagnosed to have PJI as per the MSIS Criteria. The cumulative incidence of PJI at our Institute at the end of 36 months was 1.1% (95% confidence interval [CI]: 0.59–2.91). Microorganisms were isolated by periprosthetic tissue culture (PTC) in 84 patients (sensitivity: 79% and specificity: 100%). Gram-negative aerobes were most frequently isolated (61%). Polymicrobial infections were present in 8.3% of cases. The most common infecting microorganism was *Staphylococcus aureus* (19.5%). Multidrug resistance and methicillin resistance were noted in 54% and 34% of bacterial isolates, respectively. The sensitivity and specificity of 16S rRNA PCR of periprosthetic tissue was 86% (95% CI: 74.9–89.9) and 100% (95% CI: 94.7–100), respectively. Periprosthetic tissue 16S rRNA PCR was more sensitive than PTC (P = 0.008), although both were 100% specific (P = 0.99). **Conclusions:** The incidence of PJI at our Institute compares well with other published reports. Contrary to previous reports, a predominance of Gram-negative PJI's was found. The preponderance of multidrug-resistant organisms in PJI's is worrisome. The high sensitivity and specificity of the 16S PCR assay used in our study support its use in culture-negative PJI suspected cases.

Keywords: *Aseptic failure, multidrug resistance, periprosthetic tissue, revision arthroplasty*

Introduction

Prosthetic joint infection (PJI) is a life-threatening complication of total joint arthroplasty (TJA).[1,2] Treatment of patients with PJI is challenging due to the requirement for multiple surgical procedures and long term antibiotic therapy. With an increase in the number of joint arthroplasties being performed, the incidence of PJI is likely to increase and its impact on both patients and the health care system will be enormous.

The current diagnostic algorithm for PJI includes a combination of clinical and laboratory findings, culture of periprosthetic tissue, histological examination of intraoperative specimens and imaging techniques.[3] However, reliance on these methods alone may lead to an inaccurate estimation of the true incidence of PJI.

Conventional periprosthetic tissue cultures (PTCs) have low sensitivity. Prior antibiotic administration, the presence of viable but uncultivable organisms, slow growing organisms and the presence of biofilms are some of the factors which negatively influence the sensitivity of culture results.[4]

Molecular methods using broad-range polymerase chain reaction (PCR) and sequencing of PCR amplicons have been applied to PJI, to increase the diagnostic yield.[5] However, the contribution of molecular methods for the early diagnosis

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of PJI in routine clinical practice in this region remains to be defined.

Management of PJI requires appropriate antibiotics based on culture reports. However, for empirical antimicrobial therapy, susceptibility data extrapolated from studies performed elsewhere is often used.[2,6] Local data on the microbiologic profile and susceptibility pattern of organisms isolated from PJI's is required to provide the basis of empirical treatment.

The aim of this study was to determine the incidence and risk factors of PJI following TJA in a cohort of patients who underwent total hip or knee arthroplasty (THA or TKA). The microbiological and antimicrobial susceptibility profile of the organisms isolated from patients with PJI was determined. We also evaluated the diagnostic potential of microbial culture versus amplification based DNA analysis for the diagnosis of PJI.

Materials and Methods

Study design

This study was designed as an ambispective cohort study and was performed in a 2500-bedded tertiary care hospital at Northern India. The Institutional ethical committee approved this study and informed consent was obtained from all patients before participation.

Study population

All patients who underwent primary THA or TKA, revision THA or TKA (including patients who were referred to our Institute for revision surgery) were enrolled from June 2013 to June 2017. For calculating the cumulative incidence of PJI, patients who underwent primary TJA at our hospital from January 2013 to December 2015 only were considered and followed up for a minimum of 1-year after surgery. However for evaluating the diagnostic potential of applied diagnostic methods, patients who underwent revision surgery for suspected PJI or aseptic loosening from our Institute, as well as referred cases, were also included.

Study definitions

- Diagnosis of PJI – PJI was identified as per the musculoskeletal infection society (MSIS) criteria[7]
- Aseptic failure (AF): AF was defined as loosening of the prosthesis in the absence of any one of the MSIS criteria
- Classification of PJI – PJI's were classified as early, delayed, and late PJI according to the onset of symptoms (<3 months, 3–12 months, >12 months)[3]
- Microbiological and molecular diagnosis – For the interpretation of microbiological results, a true positive was defined as the isolation of the same microorganism in two or more specimens.[7] The interpretation of the PCR results was adapted from the bacteriological criteria of MSIS.[7] A false-positive result was defined as the detection of a microorganism by culture or PCR in a sample from an AF case. True-negative results were defined as no microorganisms or no amplification by PCR obtained in any of the samples from an AF case. False-negative results were defined as no microorganisms isolated or no amplification by PCR in samples from patients considered as having PJI
- Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[8]

Specimen collection and culture

Three to five tissue samples were obtained from prosthesis interfaces and macroscopically suspected areas of inflammation for aerobic and anaerobic microbiologic culture.[9] The antimicrobial susceptibility testing was performed according to the Clinical Laboratory Standards Institute recommendations.[10]

Polymerase chain reaction analysis

PCR assay targeting the 16S rRNA gene was carried out in all the periprosthetic tissue samples from all patients (both infected and noninfected) and DNA was extracted from 25 mg of periprosthetic tissue using the QIAMP DNA mini kit method (Qiagen, Germany) according to the manufactures recommendations.

16S rRNA gene polymerase chain reaction

16S rRNA gene was amplified using conventional PCR with primers as described previously.[11] To eliminate the exogenous bacterial contamination and thereby to overcome false-positives, before amplification master mix containing Nuclease-free water, Taq Buffer B, $MgCl_2$ and Taq DNA polymerase were incubated for 15 min with 0.1 IU of DNase-I enzyme (DNase I RNase-free, Thermo Fisher Scientific, USA). After incubation, dNTP mix and primers were added to the final master mix. Amplifications were carried out in a master cycler gradient (Applied Biosystems, USA).

DNA sequencing reactions

The PCR amplicons were sequenced for identification by using ABI PRISM® Big Dye Terminator Cycle Sequencing Ready Reaction Kit (version 3.1). The sequences obtained were compared with those stored in GenBank databases using BLAST software (http://www.ncbi.nlm.nih.gov/blast). Identification to the species level was defined as 98% sequence similarity with the sequence having a high score.

Statistical analysis

The cumulative incidence of PJI was estimated using the Kaplan–Meier technique. The proportion of different microorganisms among the PJI were expressed as percentages. Sensitivities and specificities of both PTC and 16S PCR were analyzed using MSIS criteria as the gold standard. All statistical analyses were performed with Stata software version 14.2 (Stata Corp LLC, Texas, USA).

Results

Patient population

During the study period, a total of 1571 patients who had undergone 1398 TKAs and 825 THAs were clinically monitored for PJIs. Of the 1571 patients, four patients from which an inadequate number of intraoperative samples were received were excluded. Thus, a total of 1567 patients were enrolled for the study. Demographic data of these patients are summarized in Table 1. Of the 1567 enrolled patients, 237 patients who underwent revision surgery at our Institute for suspected infection or aseptic loosening were included for analysis.

A microorganism was identified in 91 (38%) of 237 revision surgery cases by culture and or PCR. After analysis of clinical and bacteriological findings, a definitive diagnosis of PJI was confirmed in 106 (44.7%) patients by MSIS criteria. The remaining 131 (55.3%) patients were classified as AF cases. The average age at the time of surgery was 55.2 years (range 14–85 years) and 56 (52.8%) were female. The majority of infections occurred within three months of the index arthroplasty (36%), 29% occurred between 3 and 12 months later, and 35% after 1 year. Nineteen patients (18%) with confirmed PJI received antibiotics 2-week before surgery.

Table 1: Total joint arthroplasty patients demographics (*n*=1567)

Characteristics	*n* (%)
Primary TJA done at our Institute	1406 (89.7)
Primary TJA done at other Institutes but referred to our Institute for revision TJA	161 (10.3)
Sex, *n* (%)	
Male	675 (43)
Female	892 (57)
Age (years)	
Mean	57
Range	14-92
Site of arthroplasty, *n* (%)	
Hip	823 (37)
Knee	1396 (63)
Reason for primary arthroplasty, *n* (%)	
Osteoarthritis	991 (63.2)
Bone fracture or trauma	196 (12.5)
Avascular bone necrosis	150 (9.6)
Rheumatoid arthritis	121 (7.7)
Ankylosing spondylitis	60 (3.8)
Bone tuberculosis	36 (2.3)
Malignancy	4 (0.2)
Other*	9 (0.6)
Comorbidities, *n* (%)	
Hypertension	692 (44.2)
Diabetes mellitus	280 (17.9)
Hyperthyroidism	169 (10.8)
Rheumatoid arthritis	150 (9.6)
Obesity	64 (4.08)
Smoking	48 (3.06)
Bronchial asthma	43 (2.7)
Tuberculosis	36 (2.29)
Steroid intake	29 (1.85)
Alcoholism	27 (1.72)
Malignancy	26 (1.65)
Depression	9 (0.57)

*Other reasons for primary arthroplasty include achondroplasia, developmental dysplasia of the hip, hydatid cyst, Perthes disease and Reiter's disease. TJA=Total joint arthroplasty

Magnitude of prosthetic joint infection

During the same study period, the cumulative incidence of PJI at our Institute was 1.1% with a minimum and maximum followup of 23 and 60 months, respectively [Table 2 and Figure 1]. The cumulative incidence of PJI was 1.53% in THA and 0.89% in TKA [Figure 2].

Risk factors

On univariate analysis, malignancy at the time of presentation and ankylosing spondylitis were risk factors associated with PJI [Table 3].

Microbiological cultures

A total of 857 periprosthetic tissue samples from 237 revision cases (106 confirmed PJIs and 131 AFs) were cultured. Of the 106 confirmed PJI s, the microbial culture was positive in 84 (79%) cases. The culture of periprosthetic tissue was sterile in all the 131 patients with AF.

Of the 84 PJI patients with positive microbiological findings, 77 (91.6%) had monomicrobial and 7 (8.3%) had polymicrobial infections. Of the monomicrobial infections, Gram-negative bacilli were isolated in 47 cases (61%). Of the seven polymicrobial infections, six were caused by two bacterial species and one was caused by *Candida tropicalis* and *Staphylococcus haemolyticus*. No anaerobes were isolated. Cultures remained sterile for the remaining 22

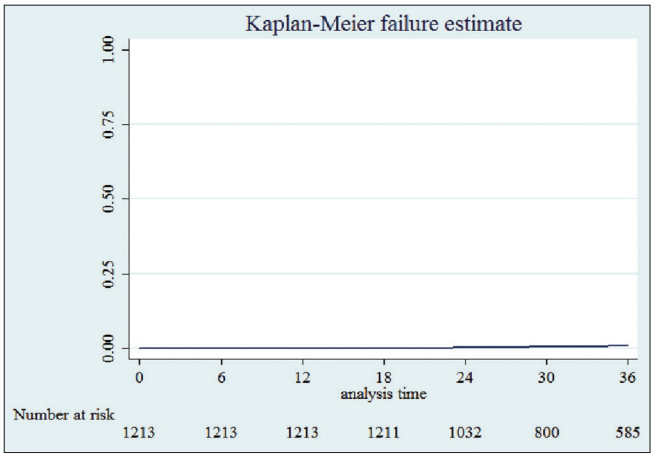

Figure 1: Kaplan–Meier estimates of the cumulative risk of infection for patients with total joint arthroplasty

Table 2: Cumulative probabilities for prosthetic joint infection

Time (months)	Cumulative risk of infection (%)								
	Hip			Knee			Total		
	Number	Risk (%)	95% CI	Number	Risk (%)	95% CI	Number	Risk (%)	95% CI
0	437	0		776	0		1213	0	
6	437	0		776	0		1213	0	
12	437	0		776	0.1	0.02-0.91	1213	0.1	0.01-0.58
18	436	0.2	0.03-1.61	775	0.1	0.02-0.91	1211	0.1	0.04-0.66
24	378	0.2	0.03-1.61	654	0.3	0.07-1.11	1032	0.3	0.08-0.8
30	298	1.1	0.42-2.95	502	0.7	0.24-1.73	800	0.8	0.41-1.63
36	215	1.5	0.63-3.7	370	0.9	0.36-2.16	585	1.1	0.59-2.1

Cumulative risk of PJI at our institute is 1.12%. The risk of infection is more in hip arthroplasty (1.53%) compared to knee arthroplasty (0.89%). CI=95% confidence interval, PJI=Prosthetic joint infection

Table 3: Univariate analysis of risk factors for prosthetic joint infection

Risk factor	Patients with PJI* (*n*=28)	Patients without PJI (*n*=1378)	*P*
Age (years)			0.437
<50	8	352	
50-64	13	521	
≥65	7	505	
Sex			0.645
Male	13	580	
Female	15	798	
Comorbidities			
Hypertension	15 (53.5)	610 (44.2)	0.327
Diabetes mellitus	6 (21.4)	237 (17.2)	0.558
Rheumatoid arthritis	2 (7.1)	129 (9.3)	0.689
Hyperthyroidism	4 (14.2)	150 (10.8)	0.568
Malignancy	3 (10.7)	20 (1.4)	0.000
Alcoholism	1 (3.5)	23 (1.6)	0.442
Obesity	2 (7.1)	56 (4.1)	0.417
Bronchial asthma	1 (3.5)	37 (2.7)	0.775
Smoking	2 (7.1)	44 (3.2)	0.245
Steroid intake	1 (3.5)	28 (2)	0.570
Tuberculosis	1 (3.5)	32 (2.3)	0.666
Ankylosing spondylitis	2 (7.1)	25 (1.8)	0.042

*Patients who developed PJI following primary total joint arthroplasty at our institute. PJI=Prosthetic joint infection

confirmed cases of PJI, including six patients (27%) being treated with antibiotics at the time of surgery.

A total of 92 isolates were detected from the intraoperative specimens of the 84 culture-positive PJI patients. The profile of the organisms isolated is detailed in Table 4. Of the total 92 isolates, 56 (61%) were Gram-negative bacteria.

The results of susceptibility studies are summarized in Table 5. Of the 56 Gram-negative isolates, MDR was noted in 36 isolates (64%). Methicillin-resistance (MR) was noted in 34% of Gram-positive isolates with all *Staphylococcus hemolyticus* isolates uniformly resistant to methicillin.

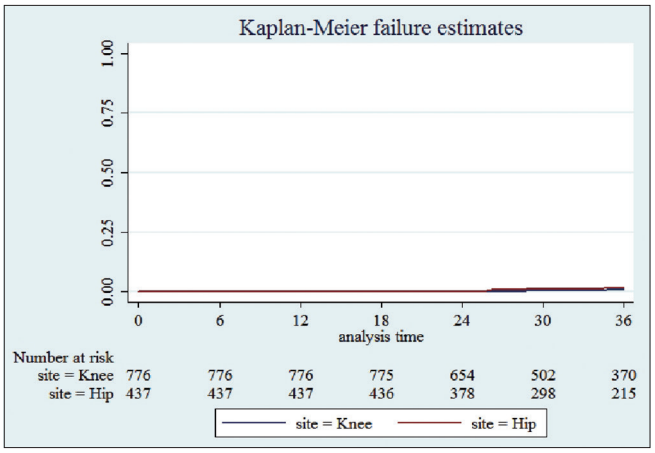

Figure 2: Kaplan–Meier estimates of the cumulative risk of infection for patients with hip and Knee arthroplasty

Analysis of 16S rRNA gene polymerase chain reaction assay and sequencing results

A total of 857 periprosthetic tissue samples from 237 revision cases (106 confirmed PJIs and 131 AF) were available for 16S rRNA gene PCR. Of the 106 confirmed PJIs, 16S rRNA gene PCR was positive in 91 (86%) cases. 16S rRNA gene PCR was negative in all the 131 AF cases.

Of the 84 confirmed PJIs with bacteriological documentation, the molecular diagnosis was also positive for all 84 PJIs (77 monomicrobial and 7 polymicrobial infections). Regarding the seven polymicrobial infections with positive molecular diagnosis, sequencing of 16S rRNA gene PCR products found one bacterium in all cases.

Of the 22 confirmed PJIs which were culture-negative, 16S rRNA gene PCR was diagnostic in seven (31.8%). Sequencing of the seven 16S rRNA amplicons identified the organisms as *Staphylococcus epidermidis* (2), *Staphylococcus haemolyticus* (2), *Staphylococcus hominis* (1), *Escherichia coli* (1), and *Lysobacter thermophilus* (1).

Table 4: Profile of microorganisms isolated from culture-positive patients with prosthetic joint infections (*n*=84)

Microorganism group	Total joint (85*)	Hip arthroplasty (*n*=51)	Knee arthroplasty (*n*=34)
Number of isolates	92 (85.9)	56 (60.8)	36 (39.1%)
Gram-positive aerobes	35 (38)	22 (38.6)	13 (36.1)
Staphylococcus aureus	18 (51.4)	15 (68.1)	3 (23.1)
Staphylococcus epidermidis	6 (17.1)	2 (9)	4 (30.7)
Staphylococcus haemolyticus	4 (11.4)	1 (4.5)	3 (23.1)
Staphylococcus lugdunensis	1 (2.8)	0	1 (7.6)
Enterococcus faecium	4 (11.4)	3 (13.6)	1 (7.6)
Enterococcus faecalis	2 (5.7)	1 (4.5)	1 (7.6)
Gram-negative aerobes	56 (60.8)	34 (59.6)	22 (61.1)
Klebsiella pneumoniae	13 (23.2)	8 (23.5)	5 (22.7)
Pseudomonas aeruginosa	12 (21.4)	5 (14.7)	7 (31.8)
Escherichia coli	12 (21.4)	9 (26.4)	3 (13.6)
Acinetobacter baumannii	8 (14.2)	6 (17.6)	2 (9.1)
Enterobacter cloacae	6 (10.7)	5 (14.7)	1 (4.5)
Pseudomonas stutzeri	1 (1.7)	0	1 (4.5)
Proteus mirabilis	1 (1.7)	1 (2.9)	0
Proteus vulgaris	1 (1.7)	0	1 (4.5)
Burkholderia cenocepacia	1 (1.7)	0	1 (4.5)
Salmonella typhimurium	1 (1.7)	0	1 (4.5)
Fungal organism			
Candida tropicalis	1 (1.1)	0	1 (2.7)

Data are *n* (%), *One patient had PJI of both the right hip and left knee. PJI=Prosthetic joint infection

Comparison between periprosthetic tissue culture and 16S rRNA polymerase chain reaction according to musculoskeletal infection society criteria

The sensitivity and specificity of 16S rRNA PCR was 86% (95% confidence interval [CI], 74.9%–89.9%) and 100% (95% CI, 94.7%–100%), respectively [Table 6]. Our results demonstrate that periprosthetic tissue 16S rRNA PCR was more sensitive than PTC (P = 0.008), although both were 100% specific (P = 0.99).

Discussion

This is the first prospective study from an Indian hospital aimed at defining the magnitude, risk factors, clinico-microbiological profile and diagnostic potential of amplification based DNA assays for the diagnosis of PJIs.

The cumulative PJI incidence at our hospital was 1.1% which is comparable with reports from other centres with high operative volume.[2,12,13] Various studies have shown that increase in the hospital operative volume results in fewer postoperative complications.[14,15] In addition, ultra-clean operating rooms, dedicated personnel, and improved surgical techniques contribute to the low incidence of PJI observed at our hospital.

Our study demonstrates the risk factors for PJI's. The increased risk of PJI in patients with malignancy not involving the index joint that was observed in the univariate analysis is a well-known risk factor.[16,17] The immunosuppressive effects of treatment for malignancy or unknown factors associated with malignancy may present opportunities for infection to develop. Patients with ankylosing spondylitis have a high rate of postoperative complications following TJA.[18,19] The use of immunosuppressive agents in the management of ankylosing spondylitis may contribute to the risk of PJI in these patients.

The culture of periprosthetic tissue is extensively used for the microbial diagnosis of PJI since it enables both the detection of the causative microorganisms and their antimicrobial susceptibility profile to guide antibiotic treatment regimens. However, culture does not have optimal sensitivity and specificity.[4] Studies have highlighted the poor specificity of culture due to the growth of contaminants when enriched media are used.[20] Bacteriological culture of single periprosthetic tissue samples has both poor sensitivity and specificity.[21] However, in our study, multiple prosthetic tissue sample culture results revealed good sensitivity and excellent specificity (79% and 100%, respectively).We used the MSIS criteria to interpret culture results which could have contributed to the increased predictive value of our culture method.

Contrary to previous reports[3,12] of the predominance of Gram-positive aerobes in PJIs, we found Gram-negative aerobic bacteria were most frequently isolated. Thus, the major aetiological agents of PJIs appear to be different in our country. These findings are important since PJIs due to Gram-negative bacteria are difficult to treat and clinical outcomes are less favorable.[22,23] The differences in the study setting and socioeconomic status of the study population from those of other studies could be a plausible reason

Table 5: Antimicrobial susceptibility pattern of aerobic bacterial isolates from prosthetic joint infection patients (*n*=84)

Antimicrobial agent (µg)	Proportion susceptible (%)				
	Staphylococcus aureus (*n*=18)	*Staphylococcus epidermidis* (*n*=6)	*Staphylococcus haemolyticus* (*n*=4)	*Staphylococcus lugdunensis* (*n*=1)	*Enterococcus* spp. (*n*=6)
Methicillin sensitive	13 (72)	5 (83)	0	1 (100)	-
Methicillin resistant	5 (28)	1 (17)	4 (100)	0	-
Amikacin (30)	17 (94)	6 (100)	1 (25)	1 (100)	-
Netilmicin (30)	17 (94)	6 (100)	1 (25)	1 (100)	-
Amoxicillin-clavum (20/10)	13 (72)	5 (83)	0	1 (100)	-
Erythromycin (15)	6 (33)	2 (33)	1 (25)	0	3 (50)
Ciprofloxacin (5)	10 (56)	3 (50)	0	1 (100)	2 (33)
Levofloxacin (5)	14 (78)	5 (83)	0	1 (100)	3 (50)
Clindamycin (2)	9 (50)	4 (67)	2 (50)	1 (100)	-
Rifampicin (5)	16 (89)	4 (67)	2 (50)	1 (100)	-
Co-trimoxazole (1.25/23.75)	15 (83)	6 (67)	2 (50)	1 (100)	-
Gentamicin (120)	-	-	-	-	3 (50)
Cefuroxime (30)	11 (61)	4 (67)	0	0	-
Multidrug resistant organisms	5 (28)	1 (17)	4 (100)	0	3 (50)

Antimicrobial agent (µg)	Proportion susceptible (%)					
	Klebsiella pneumoniae (*n*=13)	*Pseudomonas* spp. (*n*=13)	*Escherichia* coli (*n*=12)	*Acinetobacter baumannii* (n=8)	*Enterobacter* spp. (*n*=6)	*Proteus* spp. (*n*=2)
Amikacin (30)	4 (31)	4 (31)	10 (83)	2 (25)	2 (33)	0
Netilmicin (30)	4 (31)	4 (31)	10 (83)	2 (25)	2 (33)	0
Amoxicillin-clavum (20/10)	1 (8)	3 (23)	1 (8)	1 (12.5)	0	1 (50)
Ceftazidime (30)	0	5 (38)	0	1 (12.5)	0	1 (50)
Cefuroxime (30)	0	0	0	0	0	0
Cefotaxime (30)	0	5 (38)	0	1 (12.5)	0	1 (50)
Ciprofloxacin (5)	1 (8)	4 (31)	2 (17)	1 (12.5)	3 (50)	1 (50)
Levofloxacin (5)	2 (15)	4 (31)	5 (42)	3 (37.5)	4 (67)	1 (50)
Imipenem (10)	9 (69)	12 (92)	11 (92)	6 (75)	4 (67)	2 (100)
Meropenem (10)	2 (15)	5 (38)	7 (58)	2 (25)	4 (67)	1 (50)
Piperazillin-tazobactum (100/10)	5 (38)	8 (62)	9 (75)	1 (12.5)	3 (50)	2 (100)
Cefeparozone-sulbactum (75/10)	4 (31)	7 (54)	10 (83)	2 (25)	4 (67)	1 (50)
Tigecycline (15)	7 (54)		12 (100)	5 (63)	3 (50)	-
Multidrug resistant organisms	12 (92)	-8 (62)	4 (33)	6 (75)	5 (83)	1 (50)

Data are *n* (%). All *Staphylococcus* resistant to oxacillin have been considered resistant to all β-lactams; all strains of *Staphylococcus* were susceptible to vancomycin, teicoplanin and linezolid (all 30 µg). *Burkholderia cenocepacia* (1) was uniformly susceptible to the study drugs except for ceftazidime. *Salmonella typhimurium* (1) was uniformly susceptible to the study drugs except for nalidixic acid

for these differences. Recently, Benito *et al*.[24] reported a statistically significant linear increase in the proportion of PJIs caused by Gram-negative bacilli (GNB) in Spain from 2002 to 2012. The differences in the patient population, especially PJIs in old patients with severe underlying diseases could have resulted in this increasing trend in Gram-negative PJIs.

Staphylococcus aureus was the most frequent pathogen, found in 19.5% of PTC's. Majority of studies also noted a high frequency of *S. aureus* in PJIs. *K. pneumoniae* (13 isolates; 14%), *P. aeruginosa and E. coli* (12 isolates each; 13%) were isolated nearly as frequently as *S. aureus* (18 isolates; 19.5%). This is a matter of serious concern as these organisms are associated with lower infection-free rate, more surgery, and more time in hospital in treatment for PJIs.[25] In addition, *P. aeruginosa* is a known aggressive bacterium and strong biofilm producer.

S. epidermidis was the most frequent species of CoNS found in PJIs. The species distribution of CoNS in our study is in agreement with previous studies from developed countries.[3] As reported,[26,27] the association of *Salmonella typhimurium* and *Burkholderia cenocepacia* with PJI further extends the clinical spectrum of these pathogens. Although PJI caused by *Candida* is rare; nonetheless, it should be considered in symptomatic and immunocompromised patients with a joint prosthesis.[28]

Contrary to previous reports,[12,29] we did not recover any anaerobes. None of our patients had foul smelling discharge

Table 6: Comparison of culture and 16S rRNA polymerase chain reaction for diagnosis of prosthetic joint infection according to musculoskeletal infection society criteria

Results	Periprosthetic tissue culture	Periprosthetic tissue PCR
Sensitivity (%)	79.4 (CI: 70.5-86.6)	86 (CI: 74.9-89.9)
Specificity (%)	100 (CI: 97.3-100)	100 (CI: 94.7-100)
Positive predictive value (%)	100 (CI: 95.8-100)	100 (CI: 92.5-99.4)
Negative predictive value (%)	85.9 (CI: 79.4-90.9)	89 (CI: 81.8-92.2)

CI=95% confidence interval, PCR=Polymerase chain reaction

nor did they have gangrene associated with their infections. This could be responsible for the nonrecovery of anaerobes in the present study. The absence of anaerobes in PJI has also been earlier reported by Pulido *et al*. and Peel *et al*.[12,29]

The present study confirms that infections due to MDR GNB and methicillin-resistant Staphylococci are extremely common in patients with PJI. The prevalence of both MR Gram-positive organisms and MDR GNB's was higher in our population as compared to previous studies.[24] The high rates of antibiotic resistance observed in the present study may be due to the fact that ours is a tertiary care hospital with widespread use of broad-spectrum antibiotics leading to the emergence of antibiotic-resistant strains of bacteria. These findings are important for patient management.

Our study is the first study from India to evaluate the performance of 16S rRNA gene PCR for the molecular diagnosis of PJI. Clinical and bacteriological criteria were used according to the guidelines of MSIS working group for the diagnosis of PJI for validation of the 16S rRNA PCR. The PCR assays and cultures were performed on three to five tissue samples collected from each patient. The interpretation of the PCR results was adapted from the bacteriological criteria of MSIS.[7]

We found that PCR of periprosthetic tissue had significantly higher sensitivity and better NPV than culture. 16S r RNA gene PCR has been evaluated for the diagnosis of PJI and has shown a wide range of sensitivity and specificity values, from 16% to 99% and from 73% to 100%, respectively.[30-33] In the present study, the sensitivity of PCR was 86% and helped in establishing the etiology of PJI in seven (31%) culture-negative patients. Tarabichi *et al*.[32] evaluated the potential of next-generation sequencing (NGS) in the diagnosis of PJI and culture-negative PJI (CN-PJI) in particular and concluded that NGS may be a useful adjunct in the identification of causative organism(s) in CN-PJI. However, in their study, no molecular methods were concurrently tested to make comparisons with other techniques. Although a promising technique the high cost of NGS limits its use for the diagnosis of PJI in resource-limited countries.

PCR failed to identify the causative organism in 15 culture-negative patients, including six patients with a history of prior antimicrobial use highlighting the lack of sensitivity of the 16S rRNA gene PCR in antibiotic-treated patients. This is contrary to the findings of Vandercam *et al*.[34] who observed that DNA-based methods can be used to diagnose PJIs especially in patients with recent or concomitant antibiotic therapy. Possible explanations for the negative PCR results could be the presence of PCR inhibitors in tissue samples or due to the PCR inhibition caused by excessively high DNA concentrations.

A major limitation of 16S PCR is its lack of specificity due to the presence of bacterial DNA in the PCR reagents.[35,36] To improve the specificity of our PCR special attention was paid to sample collection, processing and elimination of exogenous bacterial DNA by DNase treatment, which could be present in the polymerase, as previously reported.[37] The inclusion of DNase treatment in our PCR protocol to address exogenous bacterial DNA and use of strict criteria for the interpretation of the PCR results resulted in a significantly better specificity of our PCR as compared to previous reports[31,32] where false–positive results have been reported frequently.

16S rRNA gene PCR assay followed by sequencing can also help detect rarely described human pathogens and previously identified bacteria never reported in human infection.[38] We also detected the first case of PJI caused by *Lysobacter thermophilus*.[39]

Another potential benefit of 16S gene PCR followed by sequencing is the identification of all bacteria involved in the polymicrobial infections, which is a time-consuming using conventional culture method.[40] Unfortunately, in the present study, all the seven polymicrobial infections tested positive for only one species. The difficulty of detecting mixed infections by 16S gene PCR has already been reported by Drancourt *et al*.[41] Our data corroborate current observations and highlight the use of the molecular method to complement the culture methods to improve the diagnosis of PJI.

This study had certain limitations. The cumulative incidence of PJI was calculated by analyzing the TJA data of 3 years with a minimum followup of 1.9 years thereby limiting the statistical power. All of the observations were derived from a single hospital in a specific geographic location, and therefore, may only reflect local trends of antimicrobial resistance not generalizable to other Institutions.

Conclusions

The increasing demand for TJA emphasizes the importance of ensuring the quality of patient care through the timely diagnosis of complications like PJI's. In the present study, most PJI's were caused by GNB's. This highlights the need for continuous monitoring of the local epidemiology of PJI and evaluation of its antibiotic susceptibility pattern for

successful antimicrobial therapy. The high sensitivity and specificity of the 16S PCR assay used in our study support its use in culture-negative PJI suspected cases. Although financial constraints may impede the use of amplification-based assays in our country, the morbidity and mortality associated with undiagnosed PJI's must be kept in mind before restricting their use.

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Conflicts of interest

There are no conflicts of interest.

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