Influence of 16S ribosomal RNA gene polymerase chain reaction and sequencing on antibiotic management of bone and joint infections

B Alraddadi MD[1], S Al-Azri MD[1,2], KR Forward MD[1,2]

B Alraddadi, S Al-Azri, KR Forward. Influence of 16S ribosomal RNA gene polymerase chain reaction and sequencing on antibiotic management of bone and joint infections. Can J Infect Dis Med Microbiol 2013;24(2):85-88.

INTRODUCTION: Amplification of the 16S ribosomal RNA gene by polymerase chain reaction (PCR) followed by analysis of generated sequences can be an important adjunct to conventional cultures.
OBJECTIVE: To determine how the results of this approach influence physicians' decisions regarding the management of bone and joint infections.
METHOD: Clinical and laboratory findings of patients seen at the Queen Elizabeth II Health Sciences Centre (Halifax, Nova Scotia) between December 2005 and September 2009 were reviewed. Patients who had negative cultures but likely or possible bone and joint infections were further evaluated using 16S rRNA PCR. The impact of the 16S rRNA PCR result on antibiotic management was evaluated and it was assessed whether untreated patients with negative 16S rRNA PCR subsequently presented with infections, suggesting a false-negative result.
RESULT: A total of 36 patients (mean age 62 years) were reviewed. Thirty-two patients were evaluated by infectious disease consultants; of these, 20 were considered likely to have infections. Seventeen patients were admitted with suspected prosthetic joint infections. Twenty-nine patients received antimicrobial treatment before the sample for the 16S rRNA PCR assay was obtained. Of the 36 patients, 26 (72.2%) were treated appropriately with modifications to their antibiotic regimen in response to the 16S rRNA PCR assay results. Antimicrobials were discontinued for 19 patients based on negative PCR assay and, in seven patients, antibiotics were changed based on a positive result. There were no relapses among patients with negative PCR assay in whom antibiotics were discontinued.
CONCLUSION: 16S ribosomal RNA gene PCR and sequencing is a valuable tool in the guidance of antimicrobial therapy for bone and joint infections.

Key Words: *16S PCR; rDNA; Prosthetic joint infections*

L'influence de la réaction en chaîne de la polymérase et du séquençage du gène de l'ARN ribosomique 16S sur la prise en charge antibiotique des infections osseuses et articulaires

HISTORIQUE : L'amplification de la réaction en chaîne de la polymérase (PCR) du gène de l'ARN ribosomique 16S, suivie de l'analyse des séquences générées, peut être un ajout important aux cultures habituelles.
OBJECTIF : Déterminer en quoi les résultats de cette approche influent sur les décisions des médecins à l'égard de la prise en charge des infections osseuses et articulaires.
MÉTHODOLOGIE : Les chercheurs ont analysé les résultats cliniques et de laboratoire des patients vus au *Queen Elizabeth II Health Sciences Centre* de Halifax, en Nouvelle-Écosse,entre décembre 2005 et septembre 2009. Les patients dont les cultures étaient négatives, mais qui présentaient une infection osseuse ou articulaire probable ou possible, subissaient une évaluation plus approfondie au moyen de la PCR de l'ARNr 16S. Les chercheurs ont évalué les répercussions du résultat de la PCR de l'ARNr 16S sur la prise en charge des antibiotiques et ont déterminé si les patients non traités dont la PCR de l'ARNr 16S était négative ont ensuite souffert d'infections, laissant ainsi supposer un résultat faux négatif.
RÉSULTAT : Au total, les chercheurs ont analysé le dossier de 36 patients (d'un âge moyen de 62 ans). Trente-deux patients ont été évalués par des consultants en infectiologie, et de ce nombre, 20 ont été considérés comme susceptibles d'avoir une infection. Dix-sept patients ont été hospitalisés en raison d'une présomption d'infection articulaire prosthétique. Vingt-neuf patients ont reçu un traitement antimicrobien avant l'obtention de l'échantillon en vue de la PCR de l'ARNr 16S. Des 36 patients, 26 (72,2 %) ont été traités correctement par des modifications au régime antibiotique après l'obtention des résultats de la PCR de l'ARNr 16S. Le traitement aux antimicrobiens a été interrompu chez 19 patients en raison d'une PCR négative et chez sept patients, on l'a remplacé par un autre en raison d'un résultat positif. Les chercheurs n'ont constaté aucune rechute chez les patients dont la PCR était négative et à qui on avait arrêté d'administrer des antibiotiques.
CONCLUSION : La PCR et le séquençage du gène de l'ARN ribosomique 16S est un outil précieux pour orienter le traitement antimicrobien des infections osseuses et articulaires.

The combination of 16S ribosomal RNA (rRNA) gene polymerase chain reaction (PCR) and sequencing are valuable tools in molecular microbiology (1-4). The amplification and analysis of 16S rRNA gene sequences has been implemented in many laboratories as an adjunct to conventional culture-based diagnosis. This approach is particularly useful for identification of bacteria that are fastidious or slow growing, or in circumstances in which previous antimicrobial use may lead to false-negative cultures (5,6). This approach may be especially useful in guiding the management of prosthetic joint infections (7).

Misdiagnosis of the inflamed joint can result in inappropriate treatment of an infected patient or, conversely, prolonged use of unnecessary antibiotics, adding considerably to health care costs and exposing patients to avoidable side effects.

Although the benefits and pitfalls of 16S rRNA gene PCR and sequencing have been reviewed previously (1), no study has evaluated the impact of this approach on antibiotic treatment decisions. The aim of the present study was to assess the impact of 16S rRNA gene PCR and sequencing results on antibiotic management of patients with suspected bone and joint infections.

[1]*Departments of Pathology, Microbiology and Immunology, and Medicine, Dalhousie University;* [2]*Division of Microbiology, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia*
Correspondence: Dr Kevin Forward, Division of Microbiology, Capital District Health Authority, Queen Elizabeth II Health Sciences Centre, 5788 University Avenue, Halifax, Nova Scotia B3H 1V8. Telephone 902-473-4109, fax 902-473-4432, e-mail kevin.forward@cdha.nshealth.ca

METHODS

Study population

The present analysis was a retrospective study of patients with suspected bone or joint infections who underwent 16S rRNA PCR assay between December 2005 and September 2009. Patients were seen at the Queen Elizabeth II Health Sciences Centre, an academic tertiary care medical centre located in Halifax, Nova Scotia.

Conventional bacterial cultures

Specimens were plated on 5% sheep blood agar incubated aerobically, chocolate agar incubated in carbon dioxide and brain heart infusion agar with blood incubated anaerobically. For bone and tissue biopsies, in addition to the above, macerated tissue or ground bone was cultured in thioglycollate broth and incubated for four days. If Gram-negative bacilli were apparent on Gram stain, they were plated on phenylethyl alcohol agar and MacConkey agar aerobically. All media were incubated at 35°C. Plates were examined daily for four days.

Joint fluids and biopsy samples were included in the analysis; in all cases (except one patient who had a bone biopsy positive for methicillin-resistant *Staphylococcus aureus*), culture was known to be negative for conventional bacteria. Results of all laboratory testing performed were available through the laboratory information system.

16S rRNA gene PCR and amplicon sequencing

For joint fluids, DNA was extracted from 200 µL of specimen using a QIAamp DNA Blood DNA mini kit (Qiagen Inc, Canada). For tissue or bone, DNA was extracted using a QIAamp DNA mini kit (Qiagen Inc, Canada). All extractions were performed as recommended by the manufacturers' instructions and the resulting DNA was eluted in a final volume of 100 µL. Five microlitres served as template in all reverse transcription PCR reactions.

DNA was amplified in 25 µL PCR reactions using the illustra Hot Start Mix Ready-to-Go beads (GE Healthcare, United Kingdom). Briefly, 5 µL of DNA was added to PCR reactions containing a Ready-to-Go bead, 2 mM $MgCl_2$ and 200 nM of each universal primer targeting the 16S rRNA gene. Primer combinations consisted of either 8FPL (5′-AGT TTG ATC CTG GCT CAG-3′) and 806R (5′-GGA CTA CCA GGG TAT CTA AT-3′), or 91E (5′-TCA AAK GAA TTG ACG GGG GC-3′) and 1492RPL (5′-GGT TAC CTT GTT ACG ACT T-3′). All oligonucleotides were purchased from Sigma Genosys (Canada). Thermocycling conditions were performed as follows: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, followed by a final extension for 3 min at 72°C. Amplicons were resolved using 1% agarose gel electrophoresis with ethidium bromide staining. Amplicons were excised from agarose gels and purified using a QIAquick gel extraction kit (Qiagen Inc, Canada), as recommended by the manufacturer. Sequencing was conducted using BigDye Terminator chemistry on an ABI 3130xL DNA Sequencer (Applied BioSystems, Canada) at York University (Toronto, Ontario). Sequence analysis was performed using Lasergene 7.1 Sequence Analysis Software (DNAStar, USA). The expected amplicon size using primers pair 8FPL and 806R is 834 bp. Raw sequence data were converted to FASTA format using Chromas Lite version 2.01 and overlapping sequences from the forward and reverse reactions were subjected to Basic Local Alignment Search Tool analysis (BLAST), excluding models or uncultured/environmental sample sequences. Species was assigned if the sequence yielded a query coverage of 100% (spanning a minimum of 500 bp) with an identity of 99% to 100% to a single organism. If multiple species were obtained during analysis, results were reported to genus level only. No results fell outside these criteria.

Clinical data

Charts were reviewed for age, sex, admitting service, specimen type and other relevant diagnostic tests. The presumptive (pretest) diagnosis as indicated by the infectious disease consultant, the subsequent definitive diagnosis, and whether the patient was on antibiotics before ordering 16S PCR were recorded. Changes made to antibiotics (initiation, change or discontinuation) and whether the test was ordered by infectious diseases or attending or consulting physicians were also recorded. Finally, it was determined whether untreated patients with negative 16S PCR subsequently presented with infections, suggesting a false-negative result.

The response to test results were categorized as being consistent or inconsistent with results obtained by the 16S PCR procedure results. Also recorded was whether the results of testing changed the management plan.

The present study was approved by the Capital District Health Authority Research Ethics Committee (Halifax, Nova Scotia).

RESULTS

A total of 36 patients admitted with suspected bone or joint infections were included in the present study (Table 1). The mean age was 62 years, and 19 patients were male and 17 were female. Seventeen patients were admitted because of suspected prosthetic joint infections. Thirty-three patients were evaluated by infectious disease consultants; of these, 20 were suspected to have infections. In the other patients, infection was considered unlikely but the 16S PCR test was used to definitively rule out infection. Twenty-nine patients received antimicrobial treatment before the sample for 16S PCR assay was obtained.

In total, 29 patients had a negative result and seven had a positive 16S PCR result (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Klebsiella oxytoca*, *Staphylococcus lugdunensis*, *Bacteroides* species, *Staphylococcus* species and *Streptococcus* species).

The manner in which clinicians responded to the 16S PCR results is summarized in Table 2. Twenty-six of 36 responses were consistent with the result of the 16S PCR assay. Antimicrobials were discontinued for 19 patients based on negative PCR assay and, in the case of seven patients, antimicrobials were maintained or changed in a manner consistent with the 16S PCR result.

Nine patients were continued on antibiotics despite negative PCR assay result. One patient was treated metronidazole for *Clostridium clostridioforme* that was isolated by the conventional cultures, although it was considered to be a probable contaminant by the infectious disease consultant, and one patient continued on third-generation cephalosporin for a blood culture positive for *Streptococcus bovis*.

Of the 17 patients admitted with probable prosthetic joint infections, eight were suspected to have infection by the infectious disease consultants and four of them had a positive PCR assay, which led to a change in antimicrobial therapy. Only one patient not suspected to have infection had a positive 16S PCR assay (for a coagulase-negative *Staphylococcus* species). This result was considered to be of uncertain significance and antibiotics were continued.

There were no relapses among patients with negative PCR assays in whom antibiotics were discontinued for a minimum five months follow-up.

DISCUSSION

The present study showed a definite impact on antimicrobial management based on PCR assay result from bone and joint samples. Negative PCR assay results have led to antimicrobial discontinuation even in patients clinically suspected to have infection. On the other hand, a positive PCR assay result helped in choosing the appropriate antibiotics.

It should be noted that 16S rRNA gene PCR and sequencing has some limitations. Identification of organisms by DNA sequencing is dependent on the diversity and accuracy of reference DNA sequences in the database with which they are compared (GeneBank, MicroSeq, RIDOM, Smartgene) (1). If the organism is absent from the database or its DNA sequence is misrepresented, accurate identification of the organism would be exceedingly difficult. Second, 16S rRNA gene PCR amplification usually requires that the specimen contains a single organism. Specimens containing two or more organisms would generate a mixed DNA chromatogram, hampering the analysis and interpretation of the DNA sequence. In our case, we only tested specimens that were known to be negative by conventional culture.

TABLE 1
Characteristics of patients admitted with bone and joint infections and antimicrobial therapy pre- and postpolymerase chain reaction result

Patient	Age, years/ sex	Admitting diagnosis	Pretest infectious disease opinion	Antibiotics pretest	16S result	Antibiotics post-test	Antibiotic use consistent with 16S result?
1	43/F	Osteomyelitis	Rule out	No	Negative	No antibiotic	Yes
2	36/F	Osteomyelitis	Rule in	Yes	Negative	Doxycycline, linezolid	No
3	70/F	Osteomyelitis	Rule out	Yes	Negative	Metronidazole	Yes*
4	55/M	Other noninfectious joint inflammation†	Rule in	No	Negative	No antibiotic	Yes
5	65/M	Other noninfectious joint inflammation†	Rule out	Yes	Negative	No antibiotic	Yes
6	48/F	Other noninfectious joint inflammation†	Rule out	Yes	Negative	No antibiotic	Yes
7	67/F	Prosthetic joint infection	Rule in	Yes	*Streptococcus* species	Vancomycin	Yes
8	74/M	Prosthetic joint infection	Rule out	Yes	Negative	1st-generation cephalosporin	No
9	68/F	Prosthetic joint infection	Rule out	Yes	Negative	No antibiotic	Yes
10	83/M	Prosthetic joint infection	Rule out	Yes	Negative	Metronidazole	Yes
11	59/M	Prosthetic joint infection	Rule in	No	Negative	No antibiotic	Yes
12	81/F	Prosthetic joint infection	Rule in	Yes	Negative	1st-generation cephalosporin	No
13	83/F	Prosthetic joint infection	Rule in	Yes	*Bacteroides* species	Metronidazole	Yes
14	83/M	Prosthetic joint infection	Rule out	Yes	*Staphylococcus* species	1st-generation cephalosporin	Yes
15	56/M	Prosthetic joint infection	Rule in	Yes	Negative	Vancomycin and rifampin	No
16	75/F	Prosthetic joint infection	Rule out	Yes	Negative	No antibiotic	Yes
17	63/M	Prosthetic joint infection	NA	No	Negative	No antibiotic	Yes
18	67/F	Prosthetic Joint Infection	Rule in	Yes	*Klebsiella oxytoca*	3rd-generation cephalosporin, penicillin	Yes
19	75/M	Prosthetic joint infection	Rule in	Yes	*Streptococcus pneumoniae*	Penicillin	Yes
20	56/F	Prosthetic joint infection	Rule out	Yes	Negative	1st-generation cephalosporin	No
21	51/F	Prosthetic joint infection	Rule out	No	Negative	No antibiotic	Yes
22	70/M	Prosthetic joint infection	NA	Yes	Negative	No antibiotic	Yes
23	67/M	Prosthetic joint infection	Rule in	Yes	Negative	1st-generation cephalosporin	No
24	17/M	Septic arthritis	Rule in	Yes	Negative	No antibiotic	Yes
25	57/F	Septic arthritis	Rule in	Yes	Negative	3rd-generation cephalosporin	No‡
26	42/M	Septic arthritis	Rule out	Yes	*Streptococcus pyogenes*	3rd-generation cephalosporin, penicillin	Yes
27	90/M	Septic arthritis	Rule out	Yes	Negative	No antibiotic	Yes
28	63/M	Septic arthritis	Rule in	Yes	Negative	No antibiotic	Yes
29	74/M	Septic arthritis	Rule in	Yes	Negative	FQ and rifampin	No
30	58/M	Septic arthritis	Rule in	Yes	Negative	No antibiotic	Yes
31	51/F	Septic arthritis	Rule in	Yes	Negative	No antibiotic	Yes
32	69/F	Septic arthritis	Rule in	No	Negative	Vancomycin, FQ and rifampin	No
33	18/M	Septic arthritis	Rule in	Yes	Negative	Vancomycin, FQ and rifampin	No
34	60/F	Septic arthritis	NA	No	Negative	No antibiotic	Yes
35	74/M	Septic arthritis	Rule in	Yes	Negative	No antibiotic	Yes
36	80/F	Septic arthritis	Rule in	Yes	*Staphylococcus lugdunensis*	1st-generation cephalosporin	Yes

**Patient had* Clostridium clostridioforme *superficial culture; †Gout, osteoarthritis, loose prosthesis; ‡Patient had* Streptococcus bovis *isolated from a blood culture. F Female; FQ Fluoroquinolone; M Male; NA Not seen by an infectious disease consultant*

Another limitation to PCR assay is the cost. 16S rRNA gene sequencing is more expensive than most of the traditional methods, However, when comparing the cost of hospital stay for unnecessary antimicrobial therapy and the opportunity to better direct antimicrobial management of these difficult-to-treat infections, the savings almost certainly outweigh the costs.

The sampling methods in patients with suspected prosthetic joint infection may not have been optimal because, in most cases, we received only one intraoperative sample and never received the removed prostheses in toto as recommended in recent studies (8-10). Several of the infections identified may have been identified by conventional culture if more rigorous culture approaches were used. As with culture, a positive 16S PCR result may be the result of bacterial contamination of the specimen. The analysis of multiple specimens may aid in the management of such patients; however, the regular submission of multiple clinical samples would also substantially

TABLE 2
Percentage of patients treated according to polymerase chain reaction (PCR) result

Diagnosis	Patients, n	Infection suspected according to infectious disease consultant, n	Positive according to 16S PCR, n	Antibiotic use consistent with 16S PCR result, n (%)
Prosthetic joint	17	8	5	12 (70.6)
Arthritis	13	10	2	9 (69.3)
Osteomylitis	3	1	0	2 (66.6)
Other non-infectious	3	1	0	3 (100)
Total	36	20	7	26 (72.2)

increase costs. Contamination was considered likely in one of our patients; however, the lack of certainty led to the continuation of antimicrobial treatment.

The 16S PCR assay led to identification of the infecting organisms in one-half of the patients clinically suspected to have prosthetic joint infection. Had this procedure not been performed, guided antibiotic treatment would not have been possible. Patients would have gone untreated based on negative cultures or treated with a less-effective and -appropriate antibiotic. Given the difficulty in eradicating such infections and the consequence of untreated infections, the 16S PCR assay was of considerable benefit.

Although none of the patients with a negative 16S PCR result relapsed, it should be noted that the sensitivity of 16S PCR in patients with negative conventional cultures is limited. A recent study showed that in patients with negative conventional cultures, the sensitivity of 16S PCR was 42.9%. The positive 16S PCR results were exclusively in patients who received antibiotic treatment before the test (11).

CONCLUSION

16S PCR can be a valuable tool in the management of patients with suspected bone and joint infections and negative bacterial cultures. Physicians receiving the results usually made important therapeutic choices based on the results and no patient with a negative 16S PCR result returned with a recurrent untreated infection.

REFERENCES

1. Woo PC, Lau SK, Teng JL, Tse H, Yuen KY. Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clin Microbiol Infect 2008;14:908-34.
2. Gupta R, Lanter JM, Woese CR. Sequence of the 16S ribosomal RNA from *Halobacterium volcanii*, an Archaebacterium. Science 1983;221:656-9.
3. Monstein HJ, Kihlström E, Tiveljung A. Detection and identification of bacteria using in-house broad range 16S rDNA PCR amplification and genus-specific DNA hybridization probes, located within variable regions of 16S rRNA genes. APMIS 1996;104:451-8.
4. Petti CA, Polage CR, Schreckenberger P. The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. J Clin Microbiol 2005;43:6123-5.
5. Casalta JP, Habib G, La Scola B, Drancourt M, Caus T, Raoult D. Molecular diagnosis of *Granulicatella elegans* on the cardiac valve of a patient with culture-negative endocarditis. J Clin Microbiol 2002;40:1845-7.
6. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. N Engl J Med 2004;351:1645-54.
7. Kobayashi N, Procop GW, Krebs V, Kobayashi H, Bauer TW. Molecular identification of bacteria from aseptically loose implants. Clin Orthop Relat Res 2008;466:1716-25.
8. Atkins BL, Athanasou N, Deeks JJ, et al. Prospective evaluation of criteria for microbiologic diagnosis of prosthetic joint infection at revision arthroplasty. J Clin Microbiol 1998;36:2932-9.
9. Del Pozo JL, Patel R. Clinical practice. Infection associated with prosthetic joints. N Engl J Med 2009;20:361:787-94.
10. Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. N Engl J Med 2007;357:654-63.
11. Rampini SK, Bloemberg GV, Keller PM, et al. Broad-range 16S rRNA gene polymerase chain reaction for diagnosis of culture-negative bacterial infections. Clin Infect Dis 2011;53:1245-51.