Aetiological diagnosis of brain abscesses and spinal infections: application of broad range bacterial polymerase chain reaction analysis

L Kupila, K Rantakokko-Jalava, J Jalava, S Nikkari, R Peltonen, O Meurman, R J Marttila, E Kotilainen, P Kotilainen

Objective: To evaluate the usefulness of the broad range bacterial rDNA polymerase chain reaction (PCR) method combined with DNA sequencing in the aetiological diagnosis of intracranial or spinal infections in neurological patients.

Methods: In addition to conventional methods, the broad range bacterial PCR approach was applied to examine pus or tissue specimens from cerebral or spinal lesions in patients treated in a neurological unit for a clinical or neuroradiological suspicion of bacterial brain abscess or spondylitis.

Results: Among the 44 patients with intracranial or spinal infections, the final diagnosis suggested bacterial disease in 25 patients, among whom the aetiological agent was identified in 17. A causative bacterial species was identified only by the rDNA PCR method in six cases, by both the PCR methodology and bacterial culture in six cases, and by bacterial culture alone in five. All samples in which a bacterial aetiology was identified only by the PCR approach were taken during antimicrobial treatment, and in three patients the method yielded the diagnosis even after > 12 days of parenteral treatment. One case also identified by the PCR approach alone involved a brain abscess caused by Mycoplasma hominis, which is not readily cultured by routine methods.

Conclusions: In patients with brain abscesses and spinal infections, the broad range bacterial rDNA PCR approach may be the only method to provide an aetiological diagnosis when the patient is receiving antimicrobial treatment, or when the causative agent is fastidious.

In recent years, molecular methods have increasingly been used to detect microbes in various clinical samples, including those from patients with central nervous system infections. In patients with suspected bacterial meningitis, specific polymerase chain reaction (PCR) techniques have been used for the identification of Neisseria meningitidis, Streptococcus pneumoniae, and Listeria monocytogenes or for the simultaneous detection of Neisseria meningitidis, Haemophilus influenzae, and streptococci in cerebrospinal fluid (CSF) samples. The aetiology of community acquired bacterial meningitis has also been assessed using PCR with broad range bacterial primers combined with DNA sequencing in CSF. These PCR primers are targeted at the most conserved bacterial rDNA gene sequences, rendering possible the detection of practically any bacterial species in the sample. The bacterial species can then be identified by comparing the DNA sequence of the amplification product with previously published bacterial sequence types.

PCR techniques have also been applied in the aetiological diagnostics of brain abscesses. Reports on the use of specific PCR assays in cerebral specimens from patients with brain abscesses have focused mainly on toxoplasma infections, but there are also papers describing the use of specific PCR assays to identify, for example, Entamoeba histolytica in pus from brain abscess or Mycobacterium tuberculosis in tuberculous brain lesions. One case report has described identification of Fusobacterium species in a brain abscess, and another, Streptococcus pneumoniae in a subdural empyema, by the broad range bacterial PCR approach alone.

We have previously used broad range bacterial rDNA PCR to analyse 536 clinical samples of various tissues from patients admitted to hospital during the years 1994 to 1997. That work also included samples obtained during neurosurgery, but the patients involved were not further described nor the results specifically discussed. Subsequently, the PCR method has been applied in our hospital to analyse intracranial and spinal specimens from neurological patients, when the procedure was considered clinically indicated by the attending clinicians. We describe here our experience of the value of this technique in diagnosing suspected bacterial intracranial or spinal infection at the neurological department of a university hospital in Finland.

METHODS

From the beginning of 1995 to the end of 2000, 44 pus or tissue samples from neurological patients treated at the department of neurosurgery, Turku University Hospital, Turku, Finland, were analysed by the PCR method, in addition to conventional microbiological methods. These patients were suspected of having bacterial infection in brain tissue or spinal canal.

Patients and samples

The study collection included 24 intracranial and 20 spinal samples. The intracranial pus or tissue samples were from patients operated on for a clinical or neuroradiological suspicion of brain abscess or subdural empyema, or when the intraoperative nature of the lesion remained macroscopically undefined. Eleven samples were obtained by trepanation and puncture, and 13 by stereotactic or open biopsy (table 1). The spinal samples were from patients who underwent neurosurgery for suspected spondylitis or spinal epidural abscess. The samples included five pus and 15 tissue specimens, of which 18 were obtained by biopsy during laminectomy, one tissue specimen was obtained by stereotactic biopsy, and one pus specimen was obtained by puncture (table 2).

Data were collected on the final clinical diagnoses as well as on the administration of antimicrobials and the timing of the
**Table 1** Results of analyses of specimens from 24 patients with suspected intracranial infection: broad range bacterial PCR, DNA sequencing, bacterial culture, data on operative technique and preoperative antimicrobial treatment, and final diagnosis

<table>
<thead>
<tr>
<th>No</th>
<th>Initial diagnosis</th>
<th>Operative technique</th>
<th>Intracranial specimen</th>
<th>Bacterial culture result</th>
<th>PCR</th>
<th>Sequencing result</th>
<th>Homology (%)a</th>
<th>Sequence length</th>
<th>Preoperative antibioticsb</th>
<th>Final diagnosis</th>
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<tbody>
<tr>
<td>1</td>
<td>Brain abscess</td>
<td>Puncture</td>
<td>Pus</td>
<td><em>Streptococcus equinus</em></td>
<td>+</td>
<td><em>Streptococcus intermedius</em></td>
<td>98.1</td>
<td>423</td>
<td>Yes (14 hours)</td>
<td>Brain abscess</td>
</tr>
<tr>
<td>2</td>
<td>Brain abscess or metastasis</td>
<td>Open biopsy</td>
<td>Pus</td>
<td><em>Streptococcus viridans</em></td>
<td>+</td>
<td><em>Streptococcus (sp. milleri group)</em></td>
<td>99.6</td>
<td>458</td>
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<td>Brain abscess</td>
</tr>
<tr>
<td>3</td>
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<td>Puncture</td>
<td>Pus</td>
<td><em>Streptococcus intermedius</em>/<em>anginosus</em></td>
<td>+</td>
<td><em>Streptococcus intermedius</em>/<em>anginosus</em></td>
<td>99.4</td>
<td>349</td>
<td>Yes (5 days)</td>
<td>Brain abscess</td>
</tr>
<tr>
<td>4</td>
<td>Glioblastoma</td>
<td>Open biopsy</td>
<td>Pus</td>
<td>Peptostreptococcus sp</td>
<td>+</td>
<td>Peptostreptococcus sp</td>
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<td>243</td>
<td>Yes (12 hours)</td>
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<td>Pus</td>
<td>Propionibacterium sp</td>
<td>+</td>
<td>Propionibacterium sp</td>
<td>99.7</td>
<td>299</td>
<td>No</td>
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</tr>
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<td>Puncture</td>
<td>Pus</td>
<td>Bacteroides gracilis, Peptostreptococcus, <em>Streptococcus intermedius</em></td>
<td>+</td>
<td>Bacteroides gracilis, Peptostreptococcus, <em>Streptococcus intermedius</em></td>
<td>96.2</td>
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<td>Pus</td>
<td>–</td>
<td>+</td>
<td><em>Staphylococcus aureus</em></td>
<td>98.6</td>
<td>441</td>
<td>Yes (5 days)</td>
<td>Postoperative brain abscess</td>
</tr>
<tr>
<td>8</td>
<td>Posttraumatic brain abscess</td>
<td>Puncture</td>
<td>Pus</td>
<td>–</td>
<td>+</td>
<td><em>Mycoplasma hominis</em></td>
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<td>Pus</td>
<td>–</td>
<td>+</td>
<td>Failurec</td>
<td></td>
<td></td>
<td>No</td>
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<td></td>
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<td>Fusobacterium sp</td>
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<td>–</td>
<td></td>
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<td>–</td>
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<td></td>
<td>No</td>
<td>Demyelination</td>
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<td></td>
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<td>–</td>
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<td>Brain tissue</td>
<td>Not done</td>
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<td></td>
<td></td>
<td></td>
<td>Yes (one dose)</td>
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<td>Brain tissue</td>
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<td></td>
<td>No</td>
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<td></td>
<td></td>
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<td>Stereotactic biopsy</td>
<td>Brain tissue</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>Tumour</td>
<td>Open biopsy</td>
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<td>–</td>
<td>–</td>
<td></td>
<td></td>
<td></td>
<td>Yes (one dose)</td>
<td>Mucocele</td>
</tr>
</tbody>
</table>

aThe per cent homology and the length of the overlapping sequence for the best match.
bAn effective antimicrobial treatment against the recognised microbe in the specimen; duration of preoperative antibiotic treatment is given in parentheses.
cSequencing failure was assumed if the sequencing signals reported by the instrument were weak.

DNA purification

DNA was extracted from the fresh tissue samples after proteinase K (0.1 mg/ml) digestion (56°C, two to 17 hours) with two phenol-chloroform-isooamyl alcohol extractions followed by one ether wash, as described earlier.18 Pus samples were concentrated by centrifugation20 and DNA extraction was done as described above.

PCR

The primers, reagents, and conditions used in the 23S and 16S rDNA PCR have been described previously.19 All samples were initially screened for the presence of bacterial DNA by amplification of the 23S rRNA genes with oligonucleotide primers MS 37 and MS 38.21 On the basis of sequence analysis of the 23S rDNA, these primers cover several bacterial subdivisions, as described previously.22 The bacterial DNA present in a 23S rDNA PCR positive specimen was identified by sequencing the 23S or 16S rDNA, or both. Amplification of the 23S rDNA was used in the initial screening of the samples because of its higher sensitivity compared with that of the previously described 16S rDNA PCR method. The 16S rDNA PCR product was preferred for sequencing because of the more abundant sequence data presently available. Special care was taken to avoid contamination of samples with ampiclons.23
The sequencing reactions were done as described earlier, either manually or semiautomated, by using a 373 A Stretch DNA sequencer or an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA).

### DNA sequencing

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### Sequence analysis and databases

The 16S rDNA study sequences were compared with those in a database made up of sequences obtained from GenBank, EMBL, and the ribosomal database project by using an in-house algorithm. For comparison of the 23S rDNA sequences, the FastA program was used. The interpretation

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<table>
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<th>Initial diagnosis</th>
<th>Operative technique</th>
<th>Spinal specimen</th>
<th>Bacterial culture result</th>
<th>PCR</th>
<th>Sequencing result</th>
<th>Homology (%)</th>
<th>Sequence length (nt)</th>
<th>Preoperative antibiotics</th>
<th>Blood culture result</th>
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<td>1</td>
<td>Spondylitis</td>
<td>Open biopsy</td>
<td>Pus</td>
<td>Staph aureus +</td>
<td>Staphylococcus sp, probably several species</td>
<td>86.3</td>
<td>164</td>
<td>Yes (8 days)</td>
<td>Staph aureus (–8 days)</td>
<td>Spondylitis and epidural abscess</td>
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</tr>
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<td>Open biopsy</td>
<td>Pus</td>
<td>–</td>
<td>Staphylococcus sp</td>
<td>94.7</td>
<td>216</td>
<td>Yes (15 days)</td>
<td>Staph aureus (–15 days)</td>
<td>Epidural abscess</td>
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</tr>
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<td>Staphylococcus sp</td>
<td>Staphylococcus sp</td>
<td>98.6</td>
<td>441</td>
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<td>Spondylitis</td>
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<td>Open biopsy</td>
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<td>–</td>
<td>Str intermedius</td>
<td>98.1</td>
<td>423</td>
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<td>Str mits/ gordonii/ oralis/pororis</td>
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<td>–</td>
<td>Pseudomonas sp</td>
<td>97.4</td>
<td>268</td>
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<td>Spondylitis and infective endocarditis</td>
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<td>–</td>
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<td>Staph aureus</td>
<td>Spondylitis</td>
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<td>Pseudomonas aeruginosa</td>
<td>Postoperative spondylodiscitis and epidural abscess</td>
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<td>–</td>
<td>–</td>
<td>Yes (4 days)</td>
<td>–</td>
<td>Spondylitis</td>
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<td>Bone</td>
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<td>–</td>
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<td>No</td>
<td>–</td>
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<td>–</td>
<td>–</td>
<td>Yes (10 days)</td>
<td>–</td>
<td>Spondylitis</td>
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<td></td>
</tr>
<tr>
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<td>Open biopsy</td>
<td>Disc</td>
<td>–</td>
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<td>Spondylitis</td>
<td>Open biopsy</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>Yes (8 days)</td>
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<td>Lymphoma</td>
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<td>–</td>
<td>No</td>
<td>Not done</td>
<td>Aseptic spondylitis</td>
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<td>Yes (one dose)</td>
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<td>No</td>
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<td>Aseptic spondylitis</td>
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<td>Vertebral tumour</td>
<td>Open biopsy</td>
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<td>–</td>
<td>Failure</td>
<td>–</td>
<td>Yes (one dose)</td>
<td>–</td>
<td>Lymphoma</td>
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<td>Yes (one dose)</td>
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<td>Lymphoma</td>
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<td>Yes (one dose)</td>
<td>–</td>
<td>Plasma cell granuloma</td>
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</tbody>
</table>

*The per cent homology and the length of the overlapping sequence for the best match.

*An effective antimicrobial treatment against the recognised microbe in the specimen; duration of preoperative antibiotic treatment is given in parentheses.

*The number of days a positive blood culture result was obtained before the specimen for the PCR assay and culture was taken is given in parentheses.

*An effective antimicrobial treatment against the recognised microbe in the specimen; duration of preoperative antibiotic treatment is given in parentheses.

*The sample was interpreted to contain several species if the electropherogram showed strong signals but multiple overlapping peaks in some locations.

*Negative result.

The interpretation

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The distance DNA sequencing or an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA).

### Sequence analysis and databases

The 16S rDNA study sequences were compared with those in a database made up of sequences obtained from GenBank, EMBL, and the ribosomal database project by using an in-house algorithm. For comparison of the 23S rDNA sequences, the FastA program was used. The interpretation

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of the sequencing results was based on the guidelines given by Stackebrandt and Goebel.27

Conventional microbiological methods
Samples referred for PCR analysis were at the same time also sent to the clinical microbiology laboratory of the hospital for bacterial cultures and Gram staining. For aerobic culture, the specimens were inoculated on blood agar and chocolate agar plates and incubated for a minimum of two days at 35°C in an atmosphere of 5% CO2. For anaerobic culture, the specimens were inoculated on fastidious anaerobe agar (LabM, Bury, Lancashire, UK), kanamycin-vancomycin agar, and bacteroides-bile-esculin agar plates and incubated for a minimum of four days at 35°C in an MK3 anaerobic cabinet (Don Whitley Scientific, West Yorkshire, UK). Before cultivation, biopsy specimens were homogenised in brain-heart infusion broth (Gibco BRL, Life Technologies, Paisley, Scotland). Plates were examined daily for bacterial growth. Identification of isolated colonies was based on routine microbiological methods, including the VITEK system (bioMerieux, Marcy l’Etoile, France) and different API test strips (bioMerieux).28 Gram staining was done on the specimen at the request of the attending clinician. For blood cultures, the Bactec 9240 system (Becton Dickinson, West Yorkshire, UK) was used. Before cultivation, biopsy specimens were sent to the clinical microbiology laboratory of the hospital for bacterial cultures and Gram staining. For aerobic culture, the specimens were inoculated on blood agar and chocolate agar plates and incubated for a minimum of two days at 35°C in an atmosphere of 5% CO2. For anaerobic culture, the specimens were inoculated on fastidious anaerobe agar (LabM, Bury, Lancashire, UK), kanamycin-vancomycin agar, and bacteroides-bile-esculin agar plates and incubated for a minimum of four days at 35°C in an MK3 anaerobic cabinet (Don Whitley Scientific, West Yorkshire, UK). Before cultivation, biopsy specimens were homogenised in brain-heart infusion broth (Gibco BRL, Life Technologies, Paisley, Scotland). Plates were examined daily for bacterial growth. Identification of isolated colonies was based on routine microbiological methods, including the VITEK system (bioMerieux, Marcy l’Etoile, France) and different API test strips (bioMerieux).28 Gram staining was done on the specimen at the request of the attending clinician. For blood cultures, the Bactec 9240 system (Becton Dickinson, West Yorkshire, UK) was used.

RESULTS

Intracranial specimens
The final clinical diagnoses in the 24 patients with intracranial lesions included brain abscess or subdural empyema in 14, malignant tumour in seven, and demyelination, non-specific gliosis, or mucocèle in one. Bacterial 23S rDNA PCR was positive in nine of the 14 pus samples from patients with brain abscesses or subdural empyemas. Eight of these 14 samples were positive on bacterial culture. Results of the PCR tests, DNA sequencing, and bacterial cultures of the intracranial specimens are given in table 1, as are the data on the quality of the specimens, the operative technique used, preoperative antimicrobial treatment, and the final clinical diagnoses. The specimens from patients 1, 2, 4, 5, 6, and 7 were included in our previous study.3

From six patients with brain abscesses, bacteria were detected in the specimens by both the PCR approach and bacterial culture. In five of these cases, the causative bacterial species were identified by both sequencing and culture, at least to the genus level: three cases were caused by streptococci (patients 1–3); one case by Peptostreptococcus species (patient 4); and one case by Propionibacterium acnes (patient 5). In patient 6, the brain abscess was multibacterial in aetiology—sequencing indicated the presence of several bacterial species, and bacterial culture yielded Streptococcus intermedius, Peptostreptococcus species, and Bacteroides gracilis.

Three patients with intracranial infections had specimens that were positive by PCR but negative by culture: the first had a postoperative brain abscess caused by Staphylococcus aureus, based on the sequencing result (patient 7); the second had post-traumatic brain abscesses, the specimen from which yielded Mycoplasma hominis by sequencing (patient 8); in the third case (patient 9) sequencing was not successful. In the latter, the disease was classified as probably bacterial because of the clinical features. The patient made an uneventful recovery with antimicrobial treatment.

For four patients with spondylitis or epidural abscess, causative bacteria were identified in pus samples in two cases and in vertebral bone in two cases by the PCR approach, while bacterial cultures remained negative. Staphylococcus species and Staphylococcus aureus were identified by sequencing in the samples of patients 2 and 3, respectively. These findings were in agreement with the growth of Staphylococcus aureus from the blood cultures of both patients two weeks earlier. Streptococcus intermedius was identified by sequencing in the pus specimen taken during reoperation from patient 4 with postoperative spondylodiscitis; and Streptococcus species, in the specimen from patient 5 with spondylitis.

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The PCR assay was also positive in the pus aspirate from patient 6, and sequencing of the PCR product yielded Pseudomonas species. In this patient, spondylitis and spinal abscess developed as a complication of infective endocarditis. As Streptococcus mitis growing in the blood cultures of the
patient three days before neurosurgery was the causative agent of the endocarditis, *Pseudomonas* species recognised by sequencing was designated as contamination.

Sequencing was not successful for the last PCR positive and culture negative bone specimen (patient 18). Because of the final diagnosis of lymphoma and the absence of any clinical symptoms of infection, the positive PCR finding was designated as contamination in this patient.

For two patients with spondylitis (patients 7 and 8), causative bacteria were identified from pus/bone specimens by culture while the PCR assays remained negative. One of these specimens yielded *Staphylococcus aureus*, and the other *Pseudomonas aeruginosa*. The same bacterial species were grown from the blood cultures taken simultaneously from the respective patients, confirming the aetiologic role of these pathogens in their diseases.

The tissue samples from 11 patients were negative on both PCR and culture. The final diagnosis was spondylitis/spondylodiscitis of probable bacterial origin in four of these patients (9–12). In three patients (13–15), the disease was classified as aseptic spondylitis, as one component of an autoimmune disorder of unknown origin. In the five remaining patients (16–20), spinal tumours were diagnosed by histology.

**Antimicrobial treatment and bacteriological findings**

The specimens from the above spinal bacterial (either definite or probable) infections were taken during antimicrobial treatment in eight patients. The mean duration of the preoperative treatment was 8.5 days (range two to 15 days). The causative bacteria were identified from pus or tissue specimens by the PCR approach alone in four cases, by both PCR and culture in one case, and by neither method in three cases.

**DISCUSSION**

This study was undertaken to evaluate the usefulness of the broad range bacterial PCR and sequencing in the aetiological diagnosis of infection in patients with clinically suspected intracranial or spinal infection. We are not aware of any previous reports focusing on the systematic use of the PCR and sequencing method to identify the aetiology of bacterial infection in neurosurgical patients. The results presented here are in accordance with earlier reports on patients with other infections showing that the broad range bacterial rDNA PCR approach may be the only method to yield an aetiological diagnosis in specific situations—that is, when the specimen is taken during antimicrobial treatment or when the causative agent is fastidious. In fact, all our samples with which the broad range bacterial PCR approach was the only successful investigation were taken while the patient was receiving antimicrobial treatment. Moreover, *Mycoplasma hominis* identified in one of these specimens is considered a fastidious microbe.

*Mycoplasma hominis* is a rare pathogen which normally colonises the genitourinary tract. It is not as fastidious as other mycoplasmas, and various media have yielded this organism. The few previously described cases of brain abscesses caused by *Mycoplasma hominis* in adults were diagnosed by bacterial culture. This is the first time that the organism has been identified from a brain abscess by a culture independent method. In our patient, preoperative treatment with clindamycin, which has recognised efficacy against *Mycoplasma hominis*, might also have contributed to the culture negativity of the abscess material. Although clindamycin penetrates poorly through the blood–brain barrier in healthy individuals, the situation may have been different in this patient, who had a post-traumatic brain abscess following brain contusion, leading to more effective penetration through a disrupted blood–brain barrier.

One further specimen from a brain abscess revealing *Staphylococcus aureus* was identified by the PCR method alone.

In five additional patients with brain abscesses, the aetiologic agent was identified by both the PCR approach and bacterial culture. Except for the patients described here, data on identification of the causative bacteria by 16S rDNA PCR and sequencing directly from tissue or pus specimens from brain abscess have been reported in anecdotal cases only. Also, 16S rRNA sequencing has been applied in pure cultures from brain abscesses to identify bacteria that may be difficult to classify by their phenotypic properties. Such cases include identification of *Nocardia otitidiscaiffarum* from a brain abscess in a renal transplantation patient and *Abiotrophia adiacens* from a brain abscess which had developed in a patient after neurosurgery.

Among our patients with bacterial spondylitis or epidural abscess, the causative agent was identified in pus or vertebral specimens by the PCR and sequencing method alone in four patients, two of whom also had positive blood cultures. PCR was the only method to identify the aetiological agent in the two remaining cases from whom blood cultures were not taken. It is notable that in patients with spondylitis, the PCR method could identify the microbe in the specimen even after a long course of parenteral antimicrobial treatment—among the five patients whose specimens were successfully analysed by the PCR approach, one had received parenteral antimicrobial treatment for as long as 15 days, two for at least 12 days, and one for eight days. On the other hand, five of the six specimens taken during antimicrobial treatment from spinal lesions in patients with bacterial spondylitis were negative by PCR, possibly indicating good penetration of antimicrobial agents into the spine. In our patients, blood culture proved useful as a tool to reveal the aetiology of spondylitis. In all six patients with aetiologically identified spinal infection in whom blood cultures were done, the cultures yielded corresponding pathogens.

A disadvantage associated with the application of the PCR methodology in the clinical setting is the potential for false positive results owing to cross contamination of target DNA between samples, or contamination of reagents and specimens with PCR amplicons. The use of the broad range bacterial PCR approach involves an additional risk of introducing bacterial DNA from the vessels and reagents used in various phases of sample processing and amplification. Thus rigorous measures were taken throughout the study period to avoid amplicon or sample to sample contamination and to reduce the amount of “background” bacterial DNA in the reaction. Even so, false positive amplification results may occur despite the most stringent precautions. In the present study, two positive PCR assays were considered to have been the result of contamination.

**Conclusions**

We found the broad range bacterial rDNA PCR approach to be useful in the aetiological diagnosis of infection in patients with brain abscesses or spinal infections. The results presented here show that in neurosurgical patients the PCR approach may be the only method to provide the aetiological diagnosis when the causative agent is fastidious, or when the patient is receiving antimicrobial treatment. In patients with bacterial spondylitis, this method may be successful even after >12 days of parenteral antimicrobial treatment.

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REFERENCES

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