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

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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 neurosurgical 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 neurosurgical unit for a clinical or neuroradiological suspicion of bacterial brain abscess or spondylitis.

Results: Among the 44 patients with intracranial or spinal lesions, 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 five 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. 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 samples.

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 neurosurgical 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 neurosurgical department of a university hospital in Finland.

METHODS

From the beginning of 1995 to the end of 2000, 44 pus or tissue samples from neurosurgical 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
microbiological specimens with respect to antimicrobial treatment. All positive PCR or culture results were reassessed to determine the clinical importance of the findings.

**DNA purification**

DNA was extracted from the fresh tissue samples after protease K (0.1 mg/ml) digestion (56°C, 2 to 17 hours) with two phenol-chloroform-isooamyl alcohol extractions followed by nase K (0.1 mg/ml) digestion (56°C, two to 17 hours) with two DNA was extracted from the fresh tissue samples after protei-ment. All positive PCR or culture results were reassessed to microbiological specimens with respect to antimicrobial treatment. All positive PCR or culture results were reassessed to determine the clinical importance of the findings.

**DNA purification**

DNA was extracted from the fresh tissue samples after protease K (0.1 mg/ml) digestion (56°C, 2 to 17 hours) with two phenol-chloroform-isooamyl alcohol extractions followed by one ether wash, as described earlier.93 Pu samples were concentrated by centrifugation93 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.96 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 38.97 On the basis of sequence analysis of the 23S rDNA, these primers cover several bacterial subdivisions, as described previously.96 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 amplicons.96 Strict measures were employed to separate

<table>
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<th>No</th>
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<th>Bacterial culture result</th>
<th>PCR</th>
<th>Sequencing result</th>
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</table>
The sequencing reactions were done as described earlier, or manually or semiautomatically, by using a 373 A Stretch DNA sequencer or an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA).

**DNA sequencing**

The sequencing reactions were done as described earlier, either manually or semiautomatically, by using a 373 A Stretch DNA sequencer or an ABI Prism 310 genetic analyser (Applied Biosystems, Foster City, California, USA).

### Table 2: Results of analyses of specimens from 20 patients with suspected spondylitis or epidural abscess: broad range bacterial PCR, DNA sequencing, bacterial culture, data on operative technique, preoperative antimicrobial treatment, and blood cultures, and final diagnosis

<table>
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<th>Operative technique</th>
<th>Spinal specimen</th>
<th>Bacterial culture result</th>
<th>PCR Sequencing result</th>
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<td>Staphylococcus sp</td>
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<td>Staphylococcus sp</td>
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<td>Plasma cell granuloma</td>
<td>Plasma cell granuloma</td>
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*a* The per cent homology and the length of the overlapping sequence for the best match.  
*b* An effective antimicrobial treatment against the recognised microbe in the specimen; duration of preoperative antibiotic treatment is given in parentheses.  
*c* The number of days a positive blood culture result was obtained before the specimen for the PCR assay and culture was taken are given in parentheses.  
*d* Unless otherwise indicated, blood culture was taken on the same day as the PCR.  
*e* The sample was interpreted to contain several species if the electropherogram showed strong signals but multiple overlapping peaks in some locations.  
*f* Negative result.  
*g* The patient received ceftriaxone and metronidazole.  
*h* The patient received trimethoprim prophylaxis for urinary tract infection.  
*i* Aseptic spondylitis was diagnosed as one component of an autoimmune disorder of unknown origin, as described in ref 29.  
*j* Sequencing failure was assumed if the sequencing signals reported by the instrument were weak.
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 CO2 atmosphere. For anaerobic culture, the specimens were inoculated on fastidious anaerobic 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. Blood cultures were taken if considered clinically indicated. For blood cultures, the Bactec 9240 system (Becton Dickinson, Sparks, Maryland, USA) 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 mucocele 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.

For 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 yielded post-traumatic brain abscesses, the specimen from which the patient was an alcoholic with dental caries and poor oral hygiene, which was considered to be the source of the infection. In patient 21, Propionibacterium species was grown from the third PCR negative and culture positive cerebral tissue specimen; in this patient, microbiological tests were taken as the preoperative MRI finding was suggestive of either tumour or brain abscess. On the basis of the clinical presentation and the histological finding of an astrocytoma in the tissue specimen, the culture result was designated as contamination, and the patient was not given any antimicrobial treatment.

In three patients with brain abscesses the specimens were negative by both PCR and culture. In patient 12, Aspergillus species was identified as the cause of the brain abscess at necropsy. In patient 13, Gram staining of abscess material revealed Gram positive cocci, suggesting a bacterial aetiology. In patient 14, the disease was, on clinical grounds, most probably bacterial in origin.

PCR and bacterial cultures from brain tissue specimens were negative in seven patients with non-infectious brain disease. In addition, two patients had PCR negative intracranial samples which were not referred for bacterial culture because of the high perioperative likelihood of non-infectious brain disease.

Antimicrobial treatment and bacteriological findings
The specimens from the above intracranial bacterial infections (definite or probable) were taken during antimicrobial treatment in eight patients. The mean duration of the preoperative treatment was 5.3 days (range 12 hours to 11 days). The causative bacteria were identified by both the PCR approach and culture in three cases, by PCR alone in two cases, by culture alone in one case, and by neither method in two cases.

Spinal specimens
The final clinical diagnoses of the 20 patients with spinal lesions included spondylitis or epidural abscess in 12, aseptic spondylitis in three, lymphoma in four, and plasma cell granuloma in one. Among these samples, seven were positive by PCR and three by bacterial culture. Results of the PCR tests, DNA sequencing, and bacterial cultures of the spinal specimens are given in table 2, as are the data on the quality of the specimens and the operative technique used, preoperative antimicrobial treatment, blood cultures, and final clinical diagnoses of the respective patients. The specimens from patients 1, 2, and 8 were included in our previous study.29

For patient 1 with spondylitis and epidural abscess, a spinal pus sample obtained during laminectomy yielded Staphylococcus aureus by culture and Staphylococcus species (probably several species) by sequencing. Eight days earlier, Staphylococcus aureus had been grown from the blood cultures of this patient, further confirming that this organism was the cause of her disease.

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.

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
In five additional patients with brain abscesses, the aetiologi-
ical agent was identified by both the PCR approach and bacte-
rrial culture. Except for the patients described here, data on
identification of the causative bacteria by 16S rRNA PCR and
sequencing directly from tissue or pus specimens from brain
abscesses have been reported in anecdotal cases only.14 17 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 otitidiscaviarum from a brain abscess
in a renal transplantation patient37 and Abiotrophia adiacens
from a brain abscess which had developed in a patient after
neurosurgery.18

Among our patients with bacterial spondylitis or epidural
abscess, the causative agent was identified in pus or vertebral
tissue 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 anti-
microbial 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. This might also have contributed to the culture
result of contamination.

Antimicrobial treatment and bacteriological findings
The specimens from the above spinal bacterial (either definite
or probable) infections were taken during antimicrobial treat-
mant 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 previ-
ous reports focusing on the systematic use of the PCR and
sequencing method to identify the aetiology of bacterial infec-
tion in neurosurgical patients. The results presented here are
in accordance with earlier reports on patients with other
infections showing that the broad range bacterial DNA 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.19 14 13 15 In fact, all our samples with which
the broad range bacterial PCR approach was the only success-
ful investigation were taken while the patient was receiving
antimicrobial treatment. Moreover, Mycoplasma hominis identi-
fied in one of these specimens is considered a fastidious
microbe.

Mycoplasma hominis is a rare pathogen which normally colo-
nises the genitourinary tract.1 It is not as fastidious as other
mycoplasmas, and various media have yielded this organism.22
The few previously described cases of brain abscesses caused by Mycoplasma hominis in adults were
diagnosed by bacterial culture.10–13 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 Myco-
plasma hominis,2 might also have contributed to the culture
effectiveness of the abscess material. Although clindamycin per-
ecrates poorly through the blood–brain barrier in healthy
individuals,36 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 disturbed blood–brain barrier.

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

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REFERENCES


