SHORT REPORT

Detection of *Staphylococcus aureus* by 16S rRNA directed in situ hybridisation in a patient with a brain abscess caused by small colony variants

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A 45 year old man was admitted to hospital with a right sided facial paralysis and three month history of seizures. Computed tomography showed a left temporal mass including both intracerebral and extracerebral structures. Ten years earlier the patient had undergone a neurosurgical intervention in the same anatomical region to treat a subarachnoid haemorrhage. In tissue samples and pus obtained during neurosurgery, *Staphylococcus aureus* was detected by a 16S rRNA-directed in situ hybridisation technique. Following long term cultivation, small colony variants (SCV) of methicillin resistant *S aureus* were identified. The patient was treated successfully with a combination of vancomycin and rifampin followed by prolonged treatment with teicoplanin, with no sign of infection on follow up nine months after discharge. This is the first report in which *S aureus* SCV have been identified as causative organisms in a patient with brain abscess and in which in situ hybridisation has been used to detect *S aureus* in a clinical specimen containing SCV. Antimicrobial agents such as rifampin which have intracellular activity should be included in treatment of infections caused by *S aureus* SCV.

Although small colony variants (SCV) of *Staphylococcus aureus* have been recognised for many decades, the association of this phenotype with persistent and recurrent infections has only recently been appreciated.^{1,2} The variants represent a naturally occurring slow growing subpopulation that yield very small colonies on routine media—hence their name. The small size is often due to auxotrophy for haemin or menadione, both compounds required in the biosynthesis of the electron transport chain components menaquinone and cytochromes, respectively.^{3,4} SCV form mostly non-pigmented and non-haemolytic colonies and show various other characteristics that are atypical of *S aureus*, including reduced coagulase reaction and increased resistance to aminoglycosides. Thus *S aureus* SCV are easily missed or misidentified in clinical specimens, and their significance may still be underestimated.^{1,3} In recent years, interest in the pathogenicity of *S aureus* SCV has increased with knowledge of their ability to persist for long periods inside eukaryotic cells.^{1–3,5,6}

Here, we describe what we believe to be the first case of a brain abscess caused by *S aureus* SCV. It is striking that 10 years before this abscess occurred the patient had undergone a neurosurgical intervention in the same anatomical region because of a subarachnoid haemorrhage. This is also the first report of identification of *S aureus* SCV directly from clinical specimens using in situ hybridisation.

CASE REPORT

A 45 year old man was admitted to our hospital with a right sided facial paresis and a three month history of seizures. Ten years before admission, he had had a neurosurgical operation for subarachnoid haemorrhage which had been diagnosed by cranial computed tomography (CT) (Hunt and Hess score, 2–3). This haemorrhage was caused by an aneurysm of the anterior communicating artery. Following wrapping of the aneurysm, the patient made an uneventful postoperative recovery. Because of a febrile episode at the time of this first neurosurgical intervention, he was treated with cefamandol for two weeks. There was no other medical history suggestive of any signs of acute or recurrent infection.

On admission, the patient was alert and oriented. His vital signs were normal and meningism was absent. Body temperature was normal. Neurological examination revealed an amnesic aphasia and mild hypaesthesia of the right forearm. Except for a right facial paresis, no additional paralysis was observed. A painless, non-mobile swelling was noted on clinical examination of the left temporal muscle, without signs of inflammation. On admission, the leucocyte count was $10.36 \times 10^9/l$, C reactive protein 1.0 mg/dl (normal < 0.5), and the erythrocyte sedimentation rate 5 mm/hour. Additional laboratory studies were normal, including a complete blood count, electrolytes, and coagulation profile. CT was consistent with the presence of an abscess of the left temporal muscle communicating with intracerebral structures (fig 1).

Surgical treatment

A left temporal approach was used. In the temporal musculature a cystic structure of approximately 2 cm in size was encountered, adherent to the former trepanation defect. After complete extirpation of this formation, the intracerebral part of the abscess appeared, penetrating the dura mater. When the dura mater was incised, yellowish pus spilled out of the mass. Pus and tissue specimens were obtained for microbiological testing. Granulocytes and cell detritus, but no bacteria, were observed in Gram stains done on these specimens.

Bacteriology

S aureus isolates recovered from this patient were recognised as SCV by the following characteristics:

- Gram positive cocci (detected in Gram stains from the cultivated bacteria and showing no morphological difference from *S aureus* of normal phenotype);
- pinpoint colonies following incubation on Columbia agar for 72 hours (very few pinpoint colonies had started to grow by 48 hours);
- decreased pigment formation;
- reduced haemolytic activity;
- low coagulase activity.

Figure 1 Brain abscess caused by *S aureus* small colony variants. Left: cerebral computed tomography with contrast medium; (top) intracerebral abscess and (bottom) left temporal intramuscular abscess. Right: Detection of *S aureus* cells by in situ hybridisation of a tissue section obtained from brain abscess; (top) phase contrast microscopy, (middle) in situ hybridisation using a Cy3 labelled *S aureus* specific SA-P1 probe, and (bottom) control hybridisation with a FLUOS labelled *S epidermidis* probe SEP1.

Identification of *S aureus* was based on conventional criteria (including the API Staph system (ATB32 Staph, BioMérieux, Marcy-l'Etoile, France)). SCV were confirmed as *S aureus* by testing the *S aureus* specific nuc gene, as previously described.³ Auxotrophy for haemin was tested by using standard disks, and for thymidine and menadione by impregnating disks with 15 µl of thymidine at 100 µg/l or menadione at 10 µg/ml. Following incubation on chemically defined medium and incubation for 48 hours, SCV were found to be haemin auxotrophic. Susceptibility to antimicrobial agents was determined on Mueller–Hinton agar supplemented with 2% sodium chloride following 72 hours of incubation (prolonged incubation time), and methicillin resistance was confirmed by testing for the *mecA* gene by polymerase chain reaction (PCR).⁷ Beside resistance to all β lactam antibiotics, the *S aureus* SCV isolated from this patient were not susceptible to tetracycline, kanamycin, macrolides, or fluoroquinolones. *Sma*I digests of the whole bacterial genome of all *S aureus* isolates were genotyped by pulsed field gel electrophoresis and found to be clonal, despite large differences in the colony phenotype (data not shown).

In addition, a tissue sample obtained during surgery was tested by an in situ hybridisation method with fluorescence labelled oligonucleotide probes specific for *S aureus* 16S rRNA. The DNA probe EUB338, which is specific for all eubacteria, and the *S aureus* specific oligonucleotide probe SA-P1 were

used as previously described.⁸ A 15 µl aliquot of hybridisation solution was applied to microscope slides and incubated for three hours at 43°C. Removal of the unbound probe and washing were done at 43°C. The slides were rinsed briefly with distilled water, air dried, and mounted with Citifluor solution (Citifluor Ltd, London, UK). Fluorescence was detected by using an Axioplan microscope equipped with an epifluorescence unit (fig 1).

Subsequent course

The postoperative course was uneventful, without clinical or laboratory evidence of infection. Immediate postoperative CT proved that the ring enhancement had been eliminated. CT undertaken three months postoperatively was described as normal.

Nasal swabs taken for infection control management were negative for *S aureus*. Following the operation, antimicrobial treatment was started with a combination of intravenous meropenem (2 g/8 h), clindamycin (600 mg/8 h), and gentamicin (80 mg/8 h), along with intrathecal gentamicin twice daily. After isolation of MRSA SCV, treatment was changed to a three week course of intravenous vancomycin (1 g/12 h) and rifampin (300 mg/12 h). This was followed by a two month course of teicoplanin, 800 mg three times a week. During this treatment, the patient remained afebrile and there were no signs of infection at a nine month follow up examination.

DISCUSSION

Staphylococcus aureus has mechanisms for resisting treatment that extend beyond classic resistance to antibiotics. Small colony variants of *S aureus* have been isolated in patients whose acute infection initially seems to respond to antimicrobial treatment but relapses after long disease-free intervals, or in patients with infections that persist despite appropriate antibiotic treatment. In the last decade, the significance of these variants in recurrent and antibiotic resistant infections has been demonstrated in case reports as well as in prospective studies.^{5 6 9 10}

We believe this is the first report of a brain abscess caused by SCV. Of particular interest is the fact that our patient had undergone a neurosurgical intervention in the same anatomical region because of a subarachnoid haemorrhage 10 years before the current admission. Although there is no proof that the present isolation of SCV was connected to the neurosurgical intervention 10 years before, a link is possible. In SCV, the decrease in electron transport activity may lead to resistance to defined antimicrobial agents (*S aureus* SCV have been shown to be more resistant to aminoglycosides and β lactam antibiotics^{2 4}), and it may also provide a mechanism for persisting within host tissues.^{3 6 10} Thus the transformation of *S aureus* into the SCV phenotype is regarded as a potent strategy for protection against host defences and antibiotic treatment. The ability of SCV to persist intracellularly, combined with the relatively low virulence of SCV before they revert to the rapidly growing form, helps to explain why *S aureus* seems to be eradicated only to recur months to years later. Several patients with long disease-free periods have been described, the longest interval being 54 years.¹

A further point of interest in the present report is that the 16S rRNA-directed in situ hybridisation technique was shown for the first time to be a reliable method for detecting the SCV phenotype of *S aureus* in a clinical specimen. Thus this method can detect *S aureus* in situ irrespective of its phenotype. However, the favourable outcome in the present case also supports the importance of conventional culture techniques, as antibiotic susceptibility testing is essential for adequate antibiotic treatment.⁴ In addition, different phenotypes of bacteria will only be detected by cultivation procedures.

The optimum treatment for infections caused by *S aureus* SCV has not yet been defined. It was found that trimethoprim-sulfamethoxazole combined with rifampin was the most active therapeutic regimen in a tissue culture system where the SCV were inside endothelial cells, but more research is necessary to define the optimal treatment.^{2 4} In this patient, *S aureus* SCV were shown to be methicillin resistant and were also resistant to other classes of antibiotics. Thus following isolation of MRSA SCV, treatment with a combination of intravenous vancomycin and rifampin, which has been shown to be intracellularly active, was given for three weeks. Following discharge, treatment was continued with teicoplanin for two months.

Conclusions

Small colony variants of *S aureus* may cause brain abscesses. Because of their slow growth and atypical morphology, SCV are easily missed in the clinical laboratory. This is important,

as this subpopulation is often more resistant to antibiotics than the parent population from which they arose. In addition, the intracellular position may shield SCV from host defences and decreases their exposure to antibiotics. Thus antimicrobial agents which have intracellular activity, such as rifampin, should be included in the treatment of infections caused by *S aureus* SCV. 16S rRNA-directed in situ hybridisation is a reliable technique for detecting *S aureus*, independently of its phenotype, but cannot replace conventional culture techniques. Special efforts to detect SCV should be made when an infection is particularly resistant to treatment, persists for a long period, or fails to respond to apparently adequate antimicrobial therapy. In these situations clinicians should ask the clinical laboratory specifically to search for *S aureus* SCV.

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