CLINICAL MICROBIOLOGY

Origins of Staphylococcus epidermidis and Streptococcus oralis causing bacteraemia in a bone marrow transplant patient

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Coagulase-negative staphylococcal bacteraemia in immunocompromised patients is often associated with the use of central venous catheters, while the proposed origin of viridans streptococci causing bacteraemia in this patient group is the oral cavity. This report describes an episode of polymicrobial bacteraemia caused by Staphylococcus epidermidis and Streptococcus oralis followed by several further episodes of S. epidermidis bacteraemia in a 15-year-old boy after bone marrow transplantation. Pulsed-field gel electrophoresis (PFGE) of SmalI chromosomal DNA digests was used to compare blood culture and oral isolates of S. epidermidis and Str. oralis. The results indicated that the mouth was the source of both S. epidermidis and Str. oralis causing the first episode of bacteraemia. PFGE further demonstrated that the central venous catheter was the origin of a second strain of S. epidermidis responsible for subsequent episodes of staphylococcal bacteraemia. Both the oral mucosa and central venous lines should be considered as potential sources of organisms, including coagulase-negative staphylococci, associated with bacteraemia in immunocompromised patients.

Introduction

During the first 4 weeks following allogeneic bone marrow transplantation, recipients are particularly susceptible to bacterial infection because of profound neutropenia resulting from their conditioning regimen. Concomitantly, severe oral mucositis allows endogenous bacteria to gain access to the bloodstream, while supportive care – such as the use of central venous catheters – provides further portals of entry for development of systemic and local infection.

Coagulase-negative staphylococci (CNS) have become the most frequently isolated bacteria from blood cultures of febrile neutropenic patients. These organisms are skin commensals, regarded as opportunistic pathogens, particularly in association with the use of intravenous catheters [1, 2]. CNS adhere to the catheter polymer and produce an extracellular slime substance which forms a biofilm protecting bacteria from antibiotic activity.

Viridans streptococci comprise a significant part of the normal flora of the oral cavity and previously had few disease associations other than dental caries and, much less commonly, endocarditis. However, over the last decade these organisms have become important pathogens in neutropenic patients. Viridans streptococcal bacteraemia may have a variable course, including a severe form characterised by persistent fever, acute respiratory distress and in some instances septic shock [3, 4]. Richards et al. [5] used ribotyping to demonstrate that the mouth can be the source of these organisms, which gain access to the bloodstream via chemotherapy-induced oral mucositis.

This paper describes coagulase-negative staphylococcal and viridans streptococcal bacteraemia in a bone marrow transplant patient. To determine the source of organisms causing bacteraemia, the biotype, antibiogram and PFGE type of each isolate from blood cultures were compared with isolates of coagulase-
negative staphylococci and viridans streptococci colo-
nising the patient.

Case report
A 15-year-old boy with Philadelphia positive, chronic
myeloid leukaemia was treated with hydroxyurea from
diagnosis, followed by bone marrow transplantation
from a matched unrelated donor at 9 months from
presentation. Conditioning consisted of cyclophospha-
mide, total body irradiation and Campath IG. Graft-
versus-host disease prophylaxis consisted of cyclospor-
in and methotrexate.

On day 7 post-transplant, while neutropenic and with
severe oral mucositis, the patient became febrile.
Viridans streptococci and CNS were isolated from
blood culture. Vancomycin was added to first-line
antimicrobial therapy of amikacin plus piperacil-
lin/tazobactam. On day 11, CNS were again cultured
from blood. By day 21 the patient was apyrexial and
repeat blood cultures were negative.

Throughout the period of neutropenia (until day 23),
weekly surveillance cultures (days 4, 11 and 21)
yielded CNS from all mouth swabs and from one nose
swab (day 11). Surveillance cultures of the Hickman
line exit site, faeces and urine yielded no evidence of
CNS. After neutrophil recovery, cultures from the same
sites on day 25 yielded CNS from the mouth swab
only. Oral mucositis was most severe between days 5
and 9 and did not resolve completely until day 24.

Two more episodes of CNS bacteraemia on days 29
and 70 responded rapidly to vancomycin therapy, which
allowed the Hickman line to be retained in situ.
However, following a more acute episode of sepsicae-
ia with CNS, beginning on day 150, the line was
removed (day 153). Echocardiography performed at
regular intervals was negative throughout. After
removal of the Hickman line, CNS bacteraemia did
not recur.

Materials and methods
Identification of bacteria and antibiotic
susceptibility testing
All infecting or colonising isolates of staphylococci
and streptococci were collected and identified by the API
STAPH or Rapid ID 32 STREP method (bioMérieux,
Basingstoke), respectively. All antibiotic susceptibility
patterns except that of methicillin were determined for
each isolate with susceptibility disks according to the
method of Stokes [6] on Diagnostic Sensitivity Agar
with lysed horse blood (E & O Laboratories, Bonny-
bridge) 5%. Staphylococcus aureus NCTC 6571 was
used as the control organism. Plates were incubated for
18 h at 37°C. Antibiotics tested were (μg) vancomycin
(30), rifampicin (2), clindamycin (2), fusidic acid (10),
ciprofloxacin (1), amikacin (30) and erythromycin (5).
Methicillin susceptibility was tested with 25-μg strips
on Columbia blood agar plates incubated overnight at
30°C.

Pulsed-field gel electrophoresis (PFGE)
PFGE was performed on all isolates of coagulase-
negative staphylococci as all possessed the same
biochemical identification profile. One isolate of
viridans streptococci from the mouth and the strain
from blood culture with identical biochemical identi-
fication profiles were also compared by PFGE.

Cultures were grown overnight in brain heart infusion
broth, 0.5 ml was washed in NET buffer (10 mM Tris,
1 mM EDTA, 10 mM NaCl) and resuspended in 0.25 ml
of NET buffer. Lysozyme (1 mg), mutanolysin (100
units) and RNAase (25 μg) were added to the cell
susension and mixed with an equal volume of
SeaPlaque GTG agarose (Flowgen) 2% at 50°C. The
cell/agarose suspension was pipetted into a block
mould and allowed to solidify at 4°C. Cells were lysed
at 37°C for 2–3 h in lysis buffer (lysozyme 1 mg/ml,
RNAase 25 μg/ml, 6 mM Trizma base, 100 mM EDTA,
1 M NaCl, Brj 38 0.5%, sodium deoxycholate 0.2%,
lauryl sarcosine 0.5%) followed by a further overnight
incubation at 50°C in proteolyis buffer (proteinase K
100 μg/ml, lauryl sarcosine 1% in 0.5 M EDTA). The
blocks were washed three times for 10 min each in TE
buffer (10 mM Trizma base, 1 mM EDTA). The DNA
was digested with Smal (30 units) for 2–3 h according
to the manufacturer’s instructions and electrophoresed
in an agarose (Molecular Grade Agarose, BioRad
Laboratories) 1% gel in 0.5 × TBE buffer (44.5 mM
Trizma base, 44.5 mM boric acid, 1 mM EDTA) by
the contour-clamped homogeneous electric field (CHEF)
method with a CHEF- Mapper drive module (BioRad
Laboratories). The gels were run for 23 h with a linear
ramped pulse time of 6.75–63.8 s and stained with
ethidium bromide 1 μg/ml for 30 min. Chemicals were
purchased from Sigma UK unless otherwise indicated.

Results
All staphylococci were identified as S. epidermidis
(with the same biochemical identification profile). The
viridans streptococcal isolate from the first positive
blood culture (day 7) and two others from the mouth
swab of day 4 were identified as Str. oralis. The
biochemical identification profile of the Str. oralis
from blood was identical to that of one oral isolate.

Antibiotic susceptibility tests suggested the presence
of two strains of S. epidermidis. All isolates were
susceptible to vancomycin, rifampicin and clindamycin
and resistant to methicillin, ciprofloxacin and erythro-
mycin (Table 1). Six isolates (Table 1, antibiotic A)
were also resistant to fusidic acid and amikacin while five were susceptible (Table 1, antibiogram B).

PFGE of SmaI chromosomal DNA digests of the *S. epidermidis* isolates divided them into two distinct pattern types (pulsed-field types 1 and 2), which differed from each other by >10 bands (Fig. 1). The pulsed-field types corresponded exactly to the two different antibiograms (Table 1). The isolate of *S. epidermidis* recovered from the first positive blood culture (day 7) and all isolates from positive surveillance cultures (mouth swabs taken on days 4, 11, 21, 25 and one nose swab taken on day 11) belonged to PFGE type 1, while subsequent blood culture isolates (days 11, 29, 70, 150) and the *S. epidermidis* from the tip of the Hickman line (day 153) belonged to PFGE type 2.

The isolates of *Str. oralis* from blood and mouth, with identical biochemical identification profiles and antibiograms, were also identical by PFGE (Fig. 1).

**Discussion**

These results suggest that during a period of neutropenia with severe mucositis, the patient's mouth was the source of both *Str. oralis* and *S. epidermidis* PFGE type 1 isolates responsible for the first episode of bacteraemia. Although mucositis persisted for a further 17 days, subsequent episodes of bacteraemia were caused by *S. epidermidis* PFGE type 2, the source of which was the tip of the patient’s Hickman line. All surveillance cultures of the mouth demonstrated continued colonisation with *S. epidermidis* PFGE type 1 for several weeks. The very severe nature of mucositis during the first 10 days after bone marrow transplantation may have provided the greatest opportunity for *S. epidermidis* PFGE type 1 to gain access to the bloodstream. The oral origin of this strain was also supported by the concurrent isolation of identical strains of *Str. oralis* (by API type, antibiogram and PFGE) from both mouth and blood.

This scenario is similar to that described in 1994 when Lina et al. demonstrated that isolates of *S. epidermidis* from the throat and blood of a neutropenic patient possessed identical PFGE patterns [7].
this case, culture of the tip of the patient’s Hickman line was negative.

In the present study, after the patient’s first episode of bacteraemia, *S. epidermidis* PFGE type 2 colonised the Hickman line. After adherence to the line, subsequent production of biofilm would provide extra protection for these organisms from the effects of antimicrobial agents. *S. epidermidis* PFGE type 2 was responsible for a total of four episodes of bacteraemia before removal of the intravenous line.

In the present study, the antibiograms corresponded exactly with the PFGE types. In contrast, Lina et al. [8] demonstrated that isolates of *S. epidermidis* cultured from blood of a bone marrow transplant patient over a period of 5 months belonged to the same PFGE type, but possessed different antibiograms. This highlights the importance of molecular characterisation of strains.

In summary, this paper reports a case of bacteraemia caused by *S. epidermidis* and *Streptococcus oralis* originating in the oral cavity, followed by infection by a second strain of *S. epidermidis* from a different site. Both the oral mucosa and central venous lines should be considered as potential sources of micro-organisms, including CNS, associated with bacteraemia in immunocompromised patients.

References