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ORIGINAL ARTICLE

DNA fingerprinting analysis of coagulase negative staphylococci implicated in catheter related bloodstream infections

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Aims: The epidemiological assessment of cases of coagulase negative staphylococcal catheter related bloodstream infection.

Methods: Two hundred and thirty patients with suspected catheter related bloodstream infection were evaluated over a two year period. Central venous catheters were cultured both endoluminally and extraluminally. Peripheral blood, catheter hubs, skin entry, and skin control sites were also cultured. Pulsed field gel electrophoresis (PFGE) was used to DNA fingerprint coagulase negative staphylococci isolated from patients with presumptive catheter related bloodstream infection.

Results: Sixty cases of catheter related bloodstream infection were identified, 21 of which were attributed to coagulase negative staphylococci. Two hundred and ninety four separate isolates of coagulase negative staphylococci from the 21 cases of catheter related bloodstream infection were subjected to PFGE (mean of 14 for each case). Catheter related bloodstream infection was only confirmed by PFGE analysis in 16 of the 21 cases because in the remaining five cases peripheral blood and central venous catheter coagulase negative staphylococci isolates were different. Skin entry, control skin, and central venous catheter hub isolates matched peripheral blood isolates in six, four, and seven cases, respectively. Coagulase negative staphylococci isolates could not be cultured from the patients' own skin in seven cases of catheter related bloodstream infection. Central venous catheter lumens were colonised in all cases of catheter related bloodstream infection compared with 44–81% of cases that had positive external surface catheter tip cultures, depending on the threshold used to define significant growth.

Conclusions: Catheter related bloodstream infection as a result of coagulase negative staphylococci may be over stated in about a quarter of cases, unless a discriminatory technique is used to fingerprint isolates. No single, simplistic route of bacterial contamination of central venous catheters was identified, but endoluminal catheter colonisation is invariably present in cases of catheter related bloodstream infection.

The use of central venous catheters as a means of access for monitoring and as a route of administration of drugs has become almost mandatory in patients with serious illnesses. Infections of central venous catheters are common and coagulase negative staphylococci remain the most frequent pathogens—for example, 37% of 1267 isolates in one meta-analysis.[1] Controversy remains over the source of, and route of access by, these bacteria to the central venous catheters. Recent developments, such as catheters with antimicrobial properties, are an important advance,[2 3] but until such issues are resolved it remains unclear how best to reduce the risk of catheter related bloodstream infection.

> "Pulsed field gel electrophoresis is well recognised as the gold standard for fingerprinting coagulase negative staphylococci"

Because there are at least 33 distinct coagulase negative staphylococci species that have been identified,[4] and because methods that use phenotyping alone cannot accurately distinguish between strains of coagulase negative staphylococci, DNA fingerprinting is required to clarify the epidemiology of coagulase negative staphylococci catheter related bacterial bloodstream infection. Despite the accepted difficulties in determining the relatedness of coagulase negative staphylococci, diagnostic laboratories routinely rely on limited information from phenotypic tests to compare isolates from central venous catheters and blood to achieve a diagnosis of catheter related bloodstream infection. Antibiotic susceptibility patterns may also be used, but these lack discriminatory power, and may also falsely indicate that isolates are distinct (F Perdreau-Remington *et al*. Programme and abstracts of 8th international symposium on staphylococci and staphylococcal infections. Aix-Les-Bains, France, 1996. Abstract P301). Pulsed field gel electrophoresis (PFGE) is well recognised as the gold standard for fingerprinting coagulase negative staphylococci. Our study aimed to compare PFGE fingerprints of coagulase negative staphylococci isolated from patients with presumptive catheter related bloodstream infection. Skin coagulase negative staphylococci isolates (from the central venous catheter entry and contralateral control site), hub, and separate endoluminal and extraluminal isolates were obtained to elucidate the possible sources of infection, and to evaluate further the relative importance of endoluminal and extraluminal microbial colonisation in the aetiology of catheter related bloodstream infection.

Abbreviations: cfu, colony forming units; PFGE, pulsed field gel electrophoresis; PMSF, polymethylsulfonyl fluoride; TBE, Tris/boric acid/EDTA buffer; TE, Tris/EDTA buffer; TEN, Tris/EDTA/NaCl buffer

PATIENTS AND METHODS

Two hundred and thirty patients with clinically suspected catheter related bloodstream infection were evaluated over a two year period (1996–7). On suspicion of catheter related bloodstream infection (temperature, > 37°C; leucocytosis, > 1 × x10^9/litre, or evidence of infection at the skin entry site), central venous catheters were brushed using the endoluminal brush technique[5] (significant growth was defined as > 100 colony forming units (cfu)).[5] After central venous catheter removal, tips were cultured using the roll plate method (significant growth defined as > 15 cfu).[6] After roll plate culture the external surfaces of the catheter tips were sterilised using an alcohol swab and each catheter lumen was sampled by flushing with phosphate buffered saline and subsequent culture (significant growth defined as > 1000 cfu/ml).[7] Central venous catheter hubs were individually cultured using sterile saline moistened swabs inserted into the lumen of each hub and then inoculated on to half blood agar plates. Skin entry sites and a control skin site on the opposite side of the body were sampled with sterile saline moistened swabs over a 3 × 3 cm area and then inoculated on to half blood agar plates. Peripheral blood cultures (10 ml) were taken pre and post brushing, before central venous catheter removal, and cultured using the lysis centrifugation technique (Isolator, Oxoid, Basingstoke, UK). All plates were cultured aerobically for 24–36 hours at 37°C.

Presumptive cases of catheter related bloodstream infection were defined, on the basis of the same organism, as identified by standard phenotypic methods (colonial appearances, Gram stain, and coagulase and catalase reactions), being isolated from central venous catheter samples (any of the three methods) and peripheral blood cultures. In addition, we used API Staph to provide a phenotypic biochemical profile on 100 coagulase negative staphylococci isolates from patients with catheter related bloodstream infection.

Pulsed field gel electrophoresis

Coagulase negative staphylococci isolates from each of the sites were washed and subsequently grown in triptone yeast extract/glycine broth at 37°C overnight. A 0.5 ml aliquot of bacterial suspension was pipetted into an eppendorf tube and centrifuged at 4°C at 325 ×*g* for 10 minutes. The supernatant was poured off and the cells washed with 1 ml of 10mM Tris/50mM EDTA/1M NaCl (TEN) buffer (pH 8.0), recentrifuged, and the cells then resuspended in 125 µl of TEN buffer and placed in a 50°C water bath. The resuspended cells were incorporated into 125 µl of 1% molten Seaplaque agarose at 50°C in 0.5× 45mM Tris/45mM boric acid/1mM EDTA (TBE) buffer (pH 8.0) in two plug moulds, which were subsequently digested with 1 mg/ml lysozyme and 200 µg/ml lysostaphin for four hours at 37°C without shaking. The 20mM Tris/50mM EDTA (TE) (pH 8.0) wash buffer was removed and replaced by 1 ml of proteinase K (1 mg/ml) buffer (2.5M EDTA/20mM EGTA/1% lauroyl sarcosine) (pH 9.0), followed by overnight incubation at 50°C. The proteinase K buffer was removed and the plugs washed with 1 ml of TE wash buffer, with shaking, for 30 minutes at room temperature. The TE wash buffer was then removed and replaced with 1 ml of 1mM polymethylsulfonyl fluoride (PMSF) buffer (pH 8.0) at room temperature for 30 minutes. The PMSF was replaced by 1 ml of TE wash buffer and washed as before for 30 minutes. The TE wash buffer was removed and replaced by 5 ml TE wash buffer and washed as before. The TE wash buffer was removed and the plugs were then stored at 4°C in 1 ml TE wash buffer. Plugs were washed with 1 ml of sterile water for 5–10 minutes. The water was removed and 100 µl 1× Sma1 restriction buffer added, washed, and shaken for 30 minutes. The restriction buffer was removed, 100 µl of 1× restriction buffer containing 20 U SmaI added, and left at room temperature for 2 hours, without shaking. The buffer and enzyme were then removed and plugs rinsed with 0.5× TBE buffer. Plugs were then stored dry at 4°C.

For the electrophoresis, 1.3 g of PFGE grade agarose gel with 100 ml 0.5× TBE buffer was poured around the comb/plugs and allowed to set. Two and a half litres of 0.5× TBE buffer, cooled to 14°C, were poured into the electrophoresis tank. The set gel was then placed into the tank and the electrophoresis was carried out at 6 V/cm, at settings of 45s–90s for eight hours and 5s–25s for 12 hours. The gel was stained with ethidium bromide and visualised using ultraviolet light. The digital image of the stained gel was further analysed by a computerised system (VDS Image Master). Strains differing by more than three DNA fragment bands were considered to be distinct.

Table 1 Non-confirmed and confirmed cases of catheter related bloodstream infection

Lab. no.	Skin entry	Skin control	Hub	Brush/flush	Tip roll	Blood culture
Non-confirmed						
70/96	I	G/H	F/E	D/E*	B/C	A
10/97	C/H	F/G	E/B	E*	C/D	A/B
22/97	–	B/D	–	B/C*	B*	A
24/97	C	C/E	C/D	C/D*	C	A/B
25/97	D	D/E	–	C*	C	A/B
Confirmed						
11/96	A/B	B	B	A*	A*	A
13/96	–	*Micrococcus* spp	–	A/B*	A/B	A
24/96	–	A/C	–	A*	B	A
37/96	–	C/D	–	A/B*	A	A
52/96	A	B	–	A*	A	A
69/96	D	C	A	A/B*	A*	A
79/96	–	C	–	A/B*	A	A
82/96	E	–	C/D	A*	–	A/B
90/96	–	B/C	A	A*	A*	A
06/97	*Staphylococcus aureus*	E	D	A/B/C*	*S aureus*	A
15/97	B/E	A/D	A/C	A/B*	A/B*	A
16/97	A	C/D	Gram –ve bacillus	A/B*	A	A
20/97	A	B/C	A	A*	A	A
21/97	B	A/B	A/B	A*	A/B*	A
26/97	A	A/B	A/B	A*	A*	A
40/97	A	B/C	A	A*	A/B*	A

Isolate A represents the coagulase negative staphylococci DNA fingerprint identified in the peripheral blood of each case of catheter related bloodstream infection. Letters B–I represents other distinct isolates for each case. Letters cannot be used to compare isolates in different catheter related bloodstream infection cases.
*Coagulase negative staphylococci colony counts >1000.

RESULTS

Overall, 21 of a total of 60 cases of catheter related bloodstream infection were presumed to be the result of coagulase negative staphylococci, which represented the largest single causative organism. *Staphylococcus aureus* (16), yeasts (10), enterococci (seven), and Gram negative bacilli (six) caused the remaining cases. Two hundred and ninety four macroscopically distinct isolates of coagulase negative staphylococci from the 21 cases of catheter related bloodstream infection were subjected to PFGE (mean of 14 for each case of catheter related bloodstream infection). Of these 21 cases (as determined by phenotypic methods), only 16 were confirmed by DNA fingerprinting, according to matching peripheral blood and central venous catheters isolates (table 1). We found that isolates that were indistinguishable by PFGE yielded different API Staph biochemical profiles in nine of the tested isolates.

In seven cases, hub coagulase negative staphylococci isolates matched the (pre brushing) peripheral blood isolate, compared with six cases where isolates from the skin entry site and blood isolate matched. In all cases of confirmed coagulase negative staphylococci catheter related bloodstream infection, bacteria recovered from (pre brushing) peripheral blood cultures matched isolates from central venous catheter lumens, compared with 13 of the extraluminal surfaces. If a threshold colony count of 1000 cfu was used for the endoluminal and extraluminal techniques, endoluminal colonisation was present in all 16 cases, whereas the extraluminal colonisation was present in only seven cases.

DISCUSSION

Routinely, Gram staining, catalase, and coagulase reactions are used to confirm a coagulase negative staphylococci isolate. In addition, biotyping, antibiograms, serotyping, phage typing, and plasmid profiles can be used to compare isolates. However, although such tests can distinguish some coagulase negative staphylococci, in many cases they are poor at discriminating between strains. For example, it has been shown that identical PFGE DNA fingerprints are seen for some coagulase negative staphylococci isolates that differ by up to four antimicrobial drug susceptibilities (F Perdreau-Remington *et al*. Programme and abstracts of 8th international symposium on staphylococci and staphylococcal infections. Aix-Les-Bains, France, 1996. Abstract P301). Such observations provide a diagnostic dilemma for busy laboratories and will potentially lead to mistaken assumptions of coagulase negative staphylococci isolate relatedness. PFGE is the gold standard technique for establishing the true relatedness of bacteria such as coagulase negative staphylococci. Despite being time consuming and relatively costly, the technique can be used to fingerprint all strains of coagulase negative staphylococci. Earlier studies describing PFGE used single restriction fragment differences, but it has been recognised since that differences of up to three bands can occur within a single clone of coagulase negative staphylococci.[8] In addition, we found in our present study that biochemical profiles obtained using the API Staph test for indistinguishable PFGE isolates were frequently different, confirming the limitations of such phenotypic tests for epidemiological evaluation.

> "The study of the epidemiology and aetiology of coagulase negative staphylococci catheter related bloodstream infection requires an accurate means of identifying strain relatedness"

With up to nine distinct coagulase negative staphylococci isolates identified in one of the 21 cases (patient 70/96) and an average of four macroscopically distinct coagulase negative staphylococci isolates for each case, it is clear that the study of the epidemiology and aetiology of coagulase negative staphylococci catheter related bloodstream infection requires an accurate means of identifying strain relatedness. Our results highlight the fact that almost a quarter of presumptive cases of coagulase negative staphylococci catheter related bloodstream infection cannot be confirmed by DNA fingerprinting. We found that the proportion of true coagulase negative staphylococci catheter related bloodstream infection cases fell by 24% (five of 21 cases), reducing the confirmed incidence of catheter related bloodstream infection caused by coagulase negative staphylococci to 29% (16 of 55 cases). Other large series and reviews have stated an incidence of coagulase negative staphylococci catheter related bloodstream infection similar to the unconfirmed rate of 35% (37%, 36%, and 34%).[1 9 10] Because these studies relied on phenotypic tests to match coagulase negative staphylococci, it is likely that the quoted catheter related bloodstream infection rates are also overestimates of the true incidences. The likelihood that central venous catheters and blood cultures become contaminated by skin flora, in particular coagulase negative staphylococci, together with the numerous coagulase negative staphylococci species, stresses the importance of using discriminatory techniques to determine strain relatedness and avoid misleading results.

There is still much debate over the possible sources of pathogens in cases of catheter related bloodstream infection.[11] Some believe that bacteria migrate predominantly down the external surface of the central venous catheters, and this idea forms the basis for diagnosing catheter related bloodstream infection by the tip roll technique.[6] Others have shown that bacteria can be identified in 16% of catheter tips within 90 minutes of placement.[12] Such observations are probably explained by bacterial contamination with skin flora as the catheter is introduced through the skin, rather than as a result of bacterial migration. The epidemiological evaluation of bacteria implicated in catheter related bloodstream infection using PFGE has indicated that sampling error may explain some discrepancies between catheter and skin isolates. Because many species of staphylococci are resident in the skin at varying depths, skin swab cultures may not yield bacteria that are representative of all areas and depths of the skin.[13] Furthermore, sampling errors when choosing colonies from culture plates may affect results. In our study, in only six cases were the same coagulase negative staphylococci isolated from the central venous catheter and the skin entry site. It could be argued that the application of antiseptics and dressings at the catheter exit site may have significantly altered the original skin microflora, leading to this surprisingly low figure. We attempted to minimise sampling error by selecting all colony variants at each site, and also by culturing control skin sites in addition to skin at the central venous catheter's entry point. However, even when we compared coagulase negative staphylococci isolates from catheter and control skin sites (which were not subject to antiseptics), we only found indistinguishable strains in four of 16 cases.

Another major potential source of catheter colonisation is the hub.[14] We identified only seven of 16 cases where coagulase negative staphylococci isolates from the hub and distal catheter were indistinguishable. Decontamination of the hub is encouraged when these are manipulated, and this may explain this low correlation between hub and catheter isolates. It should also be recognised that point prevalence sampling to elucidate sources of coagulase negative staphylococci may simply miss transient carriage of bacteria at skin or hub sites. Irrespective of the route of contamination (hub or skin entry site) in cases of catheter related bloodstream infection, it is widely accepted that coagulase negative staphylococci probably originate from the patients' own skin microflora. We failed in a substantial proportion of cases (seven of 16) to recover the coagulase negative staphylococci isolate causing catheter related bloodstream infection on the patient's skin.

One possible explanation for this is that coagulase negative staphylococci can be acquired exogenously, either from health care staff or the environment.[15 16] Coagulase negative staphylococci isolated from central venous catheters were not isolated from swabs of either skin or hubs in five cases. As previously suggested, hub and/or skin decontamination may explain this observation. These results appear to refute earlier findings indicating that hub or skin surveillance cultures can accurately predict catheter related bloodstream infection.[17 18]

Failure to match intravascular catheter and peripheral blood coagulase negative staphylococci isolates with those from either the patient's skin or catheter hub raises the possibility of two further sources of infection: haematogenous seeding of the catheter and contaminated infusate. We did not examine infusates used in our study because logistically this would have been difficult to achieve. As discussed earlier, there are inherent deficiencies associated with point prevalence sampling, and the examination of the infusate recovered at the time of removal is unlikely to yield bacteria of direct relevance to those colonising intravascular section(s) of the catheter. Maki *et al* examined the infusate at the time of central venous catheter removal and found contamination, primarily by coagulase negative staphylococci, in 8.4% of cases.[3] Of those infusates from cases of catheter related bloodstream infection (n = 11), one yielded bacteria (species not stated) indistinguishable from the catheter/blood isolate, in the absence of another identifiable source. The same group identified one case of apparent haematogenous seeding of the catheter from a distant source (identity not stated).[3]

> "We attempted to minimise sampling error by selecting all colony variants at each site, and also by culturing control skin sites in addition to skin at the central venous catheters entry point"

Our present study has produced further evidence to indicate that the colonisation of the catheter lumen is important in the development of catheter related bloodstream infection. In all cases of catheter related bloodstream infection the catheter lumen was colonised, and colony counts exceeded 1000 cfu/ml. In contrast, even when we analysed the results of external catheter tip sampling using a threshold of only 15 cfu, significant growth was not present in three cases. Interestingly, in Maki's original study, in all four cases of catheter related bloodstream infection the external catheter tip culture yielded > 1000 cfu.[6] It has been proposed that increasing the growth threshold above 15 cfu for external catheter tip sampling using the roll plate method will more accurately diagnose cases of catheter related bloodstream infection.[19 20] However, when the growth threshold was increased to 1000 cfu in our study, the extraluminal culture technique failed to identify nine of the 16 true cases of coagulase negative staphylococci catheter related bloodstream infection. The duration of catheterisation may have affected the relative degrees of endoluminal and extraluminal colonisation, with prolonged placement leading primarily to endoluminal as opposed to extraluminal central venous catheter contamination. Because there is a lack of evidence to support routine central venous catheter replacement,[21] this is not practised in the study hospital. The median central venous catheter survival in the coagulase negative staphylococci catheter related bloodstream infection cases was 13 days (interquartile range, 8–39 days), and this may have influenced the measured endoluminal colonisation rate.

Catheter related bloodstream infection is the most common cause of nosocomial bacteraemia, and coagulase negative staphylococci are the most often isolated pathogens. Although routine laboratory tests used to identify coagulase negative staphylococci are of benefit in the clinical setting, the results can be inaccurate and misleading unless further assessment is carried out, in particular discriminatory fingerprinting in cases of potential catheter related bloodstream infection. Clearly, this will rarely be possible as part of a routine diagnostic service. However, the importance of accurately identifying catheter related bloodstream infection cases and their aetiologies lies in the approaches to prevent such infections. In our study, endoluminal colonisation was universally present in catheter related bloodstream infection cases, and may be pivotal to its development, irrespective of the route by which the central venous catheter becomes infected. Therefore, measures should be taken to prevent luminal colonisation of central venous catheters, and the subsequent diagnosis and treatment of catheter related bloodstream infection should focus on the catheter lumen.

Take home messages

- Coagulase negative staphylococci represented the largest single causative organism in cases of catheter related bloodstream infection (21 of a total of 60 cases)
- Catheter related bloodstream infection caused by coagulase negative staphylococci may be overestimated in about a quarter of cases when only phenotypic tests are used
- The use of an additional technique to fingerprint isolates makes the estimation of coagulase negative staphylococcal catheter related bloodstream infection more accurate
- No single, simplistic route of bacterial contamination of central venous catheters was identified, but endoluminal catheter colonisation is invariably present in cases of catheter related bloodstream infection
- The accurate identification of the organisms involved in catheter related bloodstream infections and an understanding of their aetiologies is needed to prevent the occurrence of such infections

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