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Case Report

Bacterial isolates from positive paired venous catheter and peripheral blood cultures taken during parenteral nutrition were the same species but different strains: A case report

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ABSTRACT

Objective: The same microbial species isolated from blood simultaneously drawn from a central venous catheter hub and a peripheral vein (paired blood cultures) during parenteral nutrition may be assumed to represent the same strain. This case report provides an example of this assumption being incorrect along with a comparator example of it being correct. This has implications for interpretation of differential time to positivity and differential quantitative blood cultures during investigation of suspected intraluminal intravascular catheter or cannula bloodstream infection.

Case description: Two patients ages ≥18 y prescribed parenteral nutrition each had positive paired blood cultures that had been taken for suspected catheter bloodstream infection because of temperature spikes ≥38°C. The paired *Staphylococcus epidermidis* isolates from the first patient and the paired *Enterococcus faecium* isolates from the second patient were each tested beyond routine clinical care to establish if they could be different strains. The central and peripheral isolates of *Staphylococcus epidermidis* from the first patient were different strains based on hospital-reported antibiograms, genomic DNA profiles, thermograms, and weaker growth and different sizes of colonies of the central strain compared with the peripheral strain. There were no such differences for the isolates of *Enterococcus faecium* from the second patient.

Results: The central and peripheral isolates of *Staphylococcus epidermidis* from the first patient were different strains based on hospital-reported antibiograms, genomic DNA profiles, thermograms, and weaker growth and different sizes of colonies of the central strain compared with the peripheral strain. There were no such differences for the isolates of *Enterococcus faecium* from the second patient.

Conclusion: This case report indicates consideration should be given to reporting whether bacteria have been identified at either species or strain level if differential time to positivity or differential quantitative blood cultures are used to define catheter or cannula bloodstream infection.

Introduction

Parenteral nutrition (PN) requires the use of venous access, but the internal lumen of a central venous catheter can become infected. Intraluminal intravascular catheter or cannula bloodstream infection (CBI) is a significant problem. Not only is it frequent, for example >15% adult inpatients had actual or suspected CBI in a national assessment of PN practice [1], but also it can increase length of stay, morbidity, and antibiotic use. Each CBI costs ~ £10 200 (in 2019 [2], updated from £9 900 in 2015 [3]). Despite measures that aim to prevent CBIs, such as care bundles [4], prophylactic antimicrobials [5], and use of single- rather than multilumen venous catheters where possible [6], it can still occur, so early identification is important to limit the effect.

Investigation of suspected CBI often involves blood samples taken simultaneously from the catheter hub and from a peripheral vein (paired blood cultures) to assess differential time to positivity (DTP) and differential quantitative blood cultures (DQBCs).

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Isolation of the same organism from both the central and peripheral samples with either faster growth (for DTP) or more colony-forming units (for DQBCs) from the central sample indicates CBI.

However, there is confusion as to whether isolates from paired blood cultures should be identified at the species or strain level. For example, different CBI definitions require the isolates to be the same species [7–9], same organism [10], same pathogen [11], same species with the same antibiogram [12], or indistinguishable [13]. Different definitions or interpretations could result in under- or overreported rates of CBI and make comparisons between reported rates invalid [14], and this inconsistency could be exacerbated when centers amend published definitions for local use. Because isolate identification at either the species or strain level affects interpretation of DTP or DQBCs for suspected CBI, the objective of this case report is to raise the possibility that the same species obtained from paired blood cultures for suspected CBI could represent different strains.

Case report

Two inpatients ages ≥18 y prescribed PN at a single large teaching hospital (Oxford, UK) had paired (≤24 h of each other) blood culture isolates obtained during routine investigation of suspected CBI. Local research ethics committee approval (reference 17/SC/0373) and written informed consent were obtained. Each blood culture involved a nominal 10 mL fill of one aerobic and one anaerobic bottle taken and tested according to routine hospital procedures. Absence or presence of growth was established using a BACTEC FX Blood Culture System (Becton, Dickinson and Company, Wokingham, UK), and species were identified using matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker, Coventry, UK). The Phoenix broth microdilution system (Becton, Dickinson and Company) established isolate antibiograms. Isolates were investigated beyond routine clinical care using pulsed-field gel electrophoresis (PFGE) and isothermal microcalorimetry to determine if they could be different strains. The PFGE involved macrorestriction profiling of *Sma*I-digested genomic DNA using a CHEF-DR III instrument (Bio-Rad Laboratories, Watford, UK) and the HARMONY PFGE protocol [15]. Genomic profiles were analyzed using BioNumerics version 6.0 software (Applied Maths, Sint-Martens-Latem, Belgium), and bacterial strains were classified as genetically unrelated if their PFGE profiles differed by >6 bands [16]. For the isothermal microcalorimetry, 5-μL isolate within log phase (optical density of a sample measured at a wavelength of 600 nm in a 1 cm light path (OD_{600}) of 0.301–0.721) in phosphate buffered saline (Sigma-Aldrich, Gillingham, UK) diluted to an OD_{600} of 0.1 was added to 4995-μL tryptic soy broth (Sigma-Aldrich) in a nominal 20 mL vial (total vial volume 22.2 mL). The vial was inserted into the equilibration position of a Thermal Activity Monitor (TAM 2277; TA Instruments, New Castle, DE, USA) for 30 min to reach 37°C before being lowered to the measuring position. Data were recorded using the dedicated software Digitam 4.1 (one data point every 10 s with an amplifier setting of 3,000 microwatts).

The first patient had suspected CBI due to two 38°C temperature spikes for which blood cultures were taken from a single lumen peripherally inserted central (venous) catheter (PICC) and a peripheral vein. The central and peripheral samples each consisted of one aerobic and one anaerobic blood culture bottle (four bottles in total). The central samples were taken ~ 8.5 h before the peripheral samples. The central and peripheral aerobic bottles were both positive for *Staphylococcus epidermidis* (22 h 26 min and 29 h 46 min time to positivity, respectively). No growth was detected in either anaerobic bottle. Upon PICC removal, the tip culture had no growth and there were no further temperature spikes.

The second patient had suspected CBI due to three temperature spikes ≥38°C for which blood cultures were taken from a dual-lumen PICC (red and white lumens) and a peripheral vein. One aerobic and one anaerobic blood culture bottle were filled from each of the red lumen, white lumen, and peripheral vein (six bottles in total). The peripheral samples were taken 19 min before the central samples, and all six bottles were reported positive for *Enterococcus faecium*. The red and white PICC lumen aerobic bottles both flagged positive before the peripheral aerobic bottle (12 h 24 min, 13 h 24 min, and 14 h 8 min time to positivity, respectively), and the red PICC lumen anaerobic bottle flagged positive slightly faster and the white PICC lumen anaerobic bottle slower than the peripheral anaerobic bottle (13 h 4 min, 16 h 34 min, and 13 h 8 min time to positivity, respectively). The PICC remained in situ and intravenous vancomycin commenced with no further temperature spikes.

The central and peripheral paired isolates of *S. epidermidis* from the first patient appear to be different strains whereas the *E. faecium* isolates from the second patient do not.

For the *S. epidermidis* strains, hospital-reported antibiograms differed for 2 of 18 antibiotics: the central isolate was erythromycin and tetracycline sensitive whereas the peripheral isolate was erythromycin resistant and had intermediate susceptibility to tetracycline. The central isolate was generally more challenging to culture and consisted of two colony sizes, large and small, with each size confirmed as *S. epidermidis* using MALDI-TOF mass spectrometry. In contrast, the peripheral isolate formed single-size colonies. From the PFGE analysis (Fig. 1), the large and small colony variants were very closely related, having only one visible PFGE band difference. This could be variation from a single clone that arose during infection (e.g., detection of phenotypically and genotypically divergent *S. epidermidis* colonies in specimens from the same patient have previously been reported [17]). The central and peripheral strains were genetically unrelated, with their genomic profiles differing by >6 DNA fragment bands. The isothermal microcalorimetry power time curves (Fig. 2; see Supplementary Fig. 1 for the corresponding cumulative heat curves) clearly illustrate different growth patterns between the central and peripheral isolates, in particular, the delayed and weaker growth of the central isolate. In contrast, the hospital-reported antibiograms were identical for all six *E. faecium* isolates, which were each readily cultured, producing similar-sized colonies to each other. The PFGE analysis (Fig. 1) had an identical band profile between the white PICC lumen and peripheral isolates, and the red and white PICC lumen isolates were closely related strains because they differed by four bands. The thermograms (Fig. 2) and corresponding cumulative heat curves (Supplementary Fig. 1) of the white PICC lumen and peripheral isolates were indistinguishable.

Discussion

This case report presents an *S. epidermidis* example of the same species but different strains that were isolated from positive paired blood cultures along with an *E. faecium* comparator from a second patient where the same does not apply.

Differences between the central and peripheral *S. epidermidis* isolates suggestive of different strains could be explained if ≥1 of the *S. epidermidis* isolates was a contaminant. Rapid initial detection (~7 h faster positivity) but subsequent poor growth of the central isolate (observed practically and illustrated by the Fig. 2 thermograms) could result from a high initial bacterial load of a non-pathogenic contaminant. The ~8.5 h delay between sampling of central and peripheral blood increases the likelihood that the

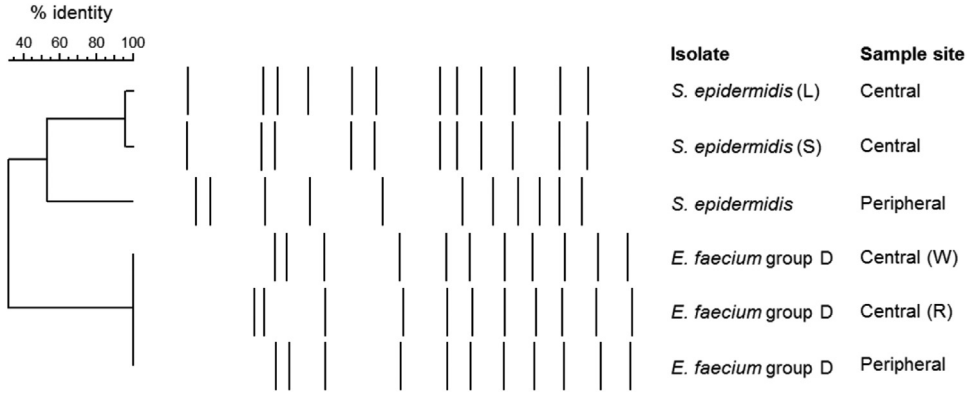

Fig. 1. PFGE genomic profiles of *Staphylococcus epidermidis* and *Enterococcus faecium* isolates obtained from paired blood cultures taken during routine clinical investigation of suspected intraluminal intravascular catheter bloodstream infection. The genomic profiles of each *S. epidermidis* colony size obtained from the same lumen are illustrated separately. The genomic profiles of *E. faecium* isolated from each lumen of a central venous catheter of a different case are illustrated separately. Strain relatedness is illustrated by a dendrogram (UPGMA type). L, large; PFGE, pulsed-field gel electrophoresis; R, red lumen; S, small; UPGMA, unweighted pair group method using arithmetic averages; W, white lumen.

central and peripheral blood samples were obtained by different operators and therefore that only one of them might be contaminated. In contrast, there was no apparent difference between the central and peripheral *E. faecium* isolates based on the same reports and investigations obtained for the *S. epidermidis* isolates. Further, it is more likely the same operator obtained all the blood samples from the second patient because they were taken much closer together (<20 min apart) compared with the blood samples taken from the first patient.

The idea that paired blood cultures could test positive for the same species while representing different strains could be important if DTP or DQBCs are used as part of a CBI definition. Not only is this consistent with a need for clinical judgment of potential sample contamination in an acute setting of suspected CBI in a PN patient but also it is relevant to surveillance reporting of infection rates. This is especially so because the infection rate numerator is expected to be low compared with the denominator (e.g., <1 CBI/1000 catheter days [18]), which means that a small difference in the number of CBIs could have a large effect on the reported rate of infection. Because the CBI rate may be used to indicate quality of care and the effect of interventions intended to reduce infection rates, consideration should be given to reporting if bacteria isolated from paired blood cultures have been tested at the strain or species level or to using a CBI definition that does not require DTP or DQBCs.

Limitations

This case report has some limitations. The time period between the central and peripheral blood samples of the pairs may meet DTP and DQBC requirements of some but not all CBI definitions, and individual sample bottles may have been filled with different quantities of blood, although that could not be ascertained because of the unknown unused bottle weights. Perhaps most importantly, because this is a case report, further reports or studies are needed before considering generalizing the need to test all blood culture samples from PN patients assessed by DTP or DQBCs at the level of strain rather than species.

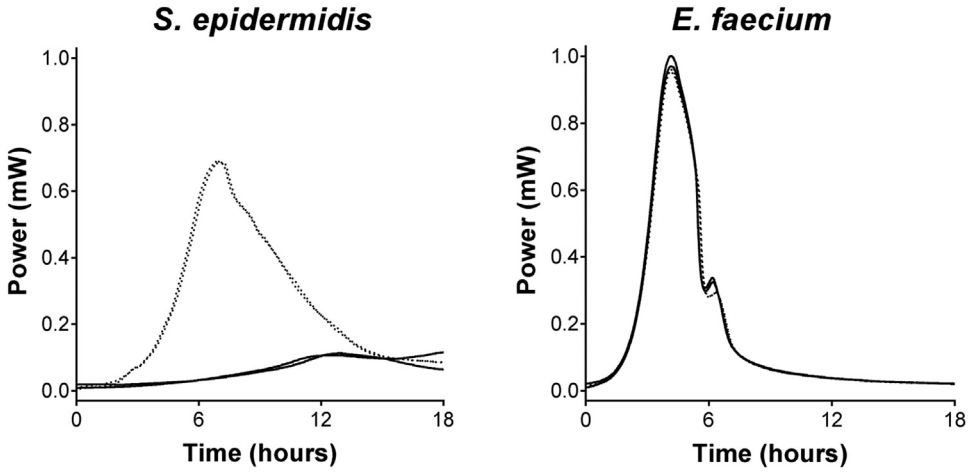

Fig. 2. Thermograms of *Staphylococcus epidermidis* (left) and *Enterococcus faecium* (right) obtained from paired blood cultures taken during routine clinical investigation of suspected intraluminal intravascular catheter bloodstream infection. The *E. faecium* isolate obtained from the dual lumen (red and white lumens) central venous catheter was taken from the white lumen. All samples were tested in duplicate in tryptic soy broth with isolates taken from central venous catheters represented by solid lines and those taken from peripheral veins represented by dotted lines. All curves were adjusted to a baseline of time (hours) = 0.

Conclusions

In this case report, paired blood cultures taken from a PN patient in routine clinical practice were positive for the same species but not the same strain. This suggests identification of isolates at the species level could overreport CBI if using a definition that requires DTP or DQBCs. Further research is necessary to evaluate the extent of this issue and help establish if it is necessary to routinely identify isolates at the strain level. In the meantime, clarity could be improved by routinely reporting if isolates assessed by DTP or DQBCs have been identified at the species or strain level.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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CRediT authorship contribution statement

Peter D. Austin: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. **Steve M. Green:** Methodology, Resources, Validation, Writing – review & editing, Investigation, Visualization. **Simon Gaisford:** Methodology, Resources, Supervision, Validation, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2024.112353.

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