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Strain relatedness in gram-negative bacteremia: Cause or contamination?

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Abstract
Aim: Bloodstream infections are a major cause of mortality, 25% of which are associated with gram-negative bacteremia. To avoid the inappropriate use of antibiotics, it is important to differentiate the bacteremia from contamination. In general, gram-positive bacteria were more likely to be contaminants than gram-negative-bacteria. There is little information in the literature concerning the epidemiology of gram-negative bacteria isolated from sequential blood cultures. Therefore, we aimed to examine the molecular epidemiology of gram-negative bacteria isolated from sequential blood cultures.
Material and Methods: A total of 56 patients (112 samples and strains) with two or more sequential positive blood cultures for gram-negative bacteria with the same antibiogram were included in the study. Pulsed-field gel electrophoresis (PFGE) and arbitrarily primed PCR (AP-PCR) were performed for the determination of strain relatedness.
Results: While PFGE analysis demonstrated relatedness in 6 isolates, AP-PCR demonstrated 9 relatedness in 112 isolates.
Discussion: The results of our study suggest that, although the possibility of contamination is very low in gram-negative bacteremia, this can still take place, as shown in sequential blood cultures with the same antibiogram.

Keywords
Bacteremia; Strain relatedness; Pulsed-field gel electrophoresis; Arbitrarily primed PCR

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Introduction

Nosocomial infections contribute to significant morbidity and mortality and occur in approximately 4% of hospitalized patients [1]. Bloodstream infection (BSI) is a major cause of mortality and healthcare costs worldwide [2]. BSI causes the prolonged length of stay in hospital and mortality in many cases. BSI is an acute event leading to life-threatening organ dysfunctions such as sepsis and septic shock [3]. Accurate diagnosis and appropriate antimicrobial treatment are essential in order to increase the survivability of the patient and to decrease high rates of morbidity and mortality [4].

The culturing of blood for pathogens is a simple procedure and provides information essential for the evaluation of a variety of infectious diseases [5]. One-fourth of sepsis cases are associated with gram-negative bacteremia [6]. Gram-negative bacilli such as Enterobacteriaceae and Pseudomonas aeruginosa are the leading causes of nosocomial bloodstream infections. Antibiotic-resistant strains have emerged among the gram-negative bacilli and are being increasingly recognized [7]. This significant increase in the incidence of infections caused by antibiotic-resistant Gram-negative bacilli has been a great concern in recent years.

To avoid the inappropriate use of antibiotics, it is important to differentiate the bacteremia from contamination. In general, gram-positive bacteria were more likely to be contaminants than were gram-negative-bacteria [8]. Appropriate antimicrobial therapy has been demonstrated to reduce mortality in Gram-negative bacteremia patients [9]. There is only a little information in the literature about the epidemiology of gram-negative bacteria isolation from sequential blood cultures. The aim of the current study was to examine the molecular epidemiology of gram-negative bacteria isolated from sequential blood cultures.

Material and Methods

Approval for the study was obtained from the institutional review board (No: 18824186-799). A total of 56 patients (112 samples and strains) with two or more sequential positive blood cultures for gram-negative bacteria that had the same antibiograms within a one-week period were included in the study. Clinical data including age, sex, underlying disease, symptoms of infection, other infection sites, and exposure to antibiotic therapy were collected.

Isolation of gram-negative bacteria from blood cultures was carried out using the Bact/Alert automated system (bioMerieux Industry, Hazelwood, MS, USA). The isolated strains were identified by the VITEK 2 system, using GN cards. All isolates were stored frozen at -70°C. Susceptibility testing for all isolates was performed by a disk-diffusion assay using antibiotic disks (Oxoid, UK) on Mueller–Hinton agar in a rich CO2 atmosphere and by VITEK 2 GNS cards. Pulsed-field gel electrophoresis (PFGE) of the samples treated with XbaIrestriction enzyme (Vivantis) was performed by means of a commercially available kit (GenePath Group 1, Bio-Rad Laboratories, Hercules, CA). Strain relatedness was defined as identical (no difference in banding), closely related (2 to 3 different bands), or unrelated (>3different bands) [10]. In addition, we used a well-known effective PCR method (arbitrarily primed PCR: AP-PCR) for genotyping of the strains. To prepare templates for AP-PCR, single colonies were inoculated into L broth and were incubated as stationary cultures at 37°C for about 16 hours. Then, 0.5 ml of the resultant stationary-phase culture was boiled for 10 min, diluted 10-fold in distilled water, and used immediately or stored at 4°C until needed. Phenol-extracted template DNA was prepared for AP-PCR. The previously described arbitrary primers (P1:5'-AAGAG CCGT; P2: 5'-CCGC GCCAA; P3:5'-AACGCGCAAC; P4:5'-GCGATCCCCA) were used [11]. Gels were photographed.

Results

Demographic data of the patients are shown in Table 1.

A total of 112 episodes of bacteremia were identified. The majority of these episodes were due to Escherichia coli (26 patients, 52 isolates), with the remainder due to Acinetobacter baumannii (19 patients, 38 isolates), Klebsiella pneumoniae (7 patients, 14 isolates) and Klebsiellaoxytoca (4 patients, 8 isolates). Sequential isolates had similar antibiotic sensitivity. Most of the strains were extended-spectrum β-lactamase positive. PFGE analysis demonstrated relatedness in 4 out of 112isolates (Figure 1). These isolates were E.coli. The remaining isolates were identical. AP-PCR analysis revealed differences in six E.coli, two K. pneumoniae and one A. baumannii isolate.

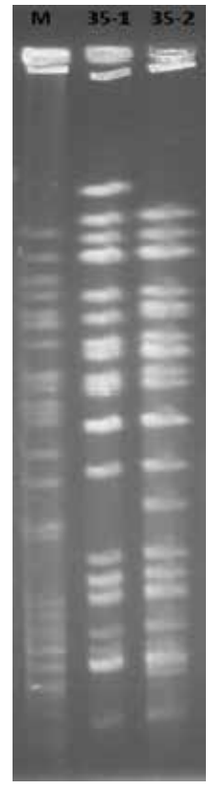

Figure 1. Pulsed field gel electrophoretic patterns of non-identical isolates. The figure shows unrelated sequential isolates of patient 35 (35-1, 35-2). M: Ladder.

Table 1. Demographic features of the patients

Mean age	48.28 ± 11.6	
Female / male	32/24	
Antibiotic therapy (yes / no)	51/5	
Source of infection	Lung	22 (%39.2)
	Abdomen	14 (%25)
	Urinary Tract	11 (%19.6)
	Wound	9 (%16)
Underlying disease	Malignancy	23
	Diabetes	19
	Other	14

Discussion

Understanding epidemiological and microbiological data is critical in order to struggle with potentially life-threatening infections such as BSI, because understanding pathogens and their resistance patterns is of great importance for successful treatment. In this study, molecular epidemiology of gram-negative bacteria isolated from sequential blood cultures was investigated. However, resistance patterns are not discussed in detail here, since they are beyond the scope of this study.

Blood culture is a critical tool for the healthcare professional, a positive blood culture can suggest a definitive diagnosis but, like any test, false positive results can limit the utility of this important tool [12]. Contamination is not uncommon during the sample preparation process, which creates serious problems for interventions. Most contaminants are probably introduced from the patient's skin during blood collection [5]. Frequent contaminants included coagulase-negative staphylococci, Bacillus species and Corynebacterium species [8]. Several clinical studies of bloodstream infections have provided guidelines for differentiating true pathogens from contaminants, but there is no gold standard for differentiating pathogens from contaminants [5].

Gram-negative bacteremia accounts for approximately 25% to 27% of bloodstream infections [13]. Gram-negative bacilli such as Enterobacteriaceaeand P. aeruginosa are the leading causes of nosocomial bloodstream infections, and appropriate antimicrobial therapy has been shown to reduce mortality among patients with gram-negative bacteremia [7]. The identification of the organism has been shown to be the most important prognostic factor in a predictive model for differentiating contamination from infection in blood culture results of bacteremia [12]. In general, gram-positive bacteria were more likely to be contaminants than gram-negative-bacteria [8]. It is accepted that E. coli and other members of the Enterobacteriaceae and P. aeruginosa always or nearly always (>90%) represent true bacteremia, which our study confirms [14].

One proven methodology that can help differentiate blood culture contamination from a true infection is the number of blood culture sets that grow organisms. For true bacteremia, multiple blood culture sets usually grow the same organism [12]. In our study, we included two or more sequential positive blood cultures for gram-negative bacteria with the same antibiograms within a one-week period. We found 4 related Escherichia coli isolates and two closely related isolates of Klebsiella (K. pneumoniae and A.baumannii).

Even though there are numerous relatedness studies on gram-positive bacteria, there are very few studies in the literature on the gram-negatives [15-21]. We believe that this is due to overlooking gram-negative bacteria as potential contaminants. In conclusion, the results of our study suggest that although the possibility of contamination is very low in gram-negative bacteremia, it can still take place even in sequential blood cultures with the same antibiograms. This must be taken into account in bacteriemia, especially when antibiotic therapy fails.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

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