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Determination of clinical significance of coagulase-negative staphylococci in blood cultures

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ABSTRACT

The aim of this study was to investigate the criteria used to distinguish coagulase-negative staphylococci (CoNS) bacteremia from contamination. We evaluated 162 adult patients with CoNS-positive blood cultures (BCs). Of the 162 patients, 35 (21.6%) had at least 2 positive BCs and 127 (78.4%) had a single positive BC. According to the Laboratory-Confirmed Bloodstream Infection (LCBI) criteria, 24 (14.8%) patients with the same species of CoNS had true bacteremia, and 138 (85.2%) patients had contaminants. Despite the detection of the same CoNS species, 9 of the 24 patients had different CoNS genotypes. Using clinical assessments, only 20 patients were diagnosed with true bacteremia, 8 of them had a single positive BC. We concluded that only using the LCBI criteria or clinical evaluations of a patient were not sufficient to distinguish CoNS bacteremia from contamination. Molecular identification should also be performed as a diagnostic laboratory parameter for CoNS bacteremia.

1. Introduction

Coagulase-negative staphylococci (CoNS) are the most common isolated microorganisms from blood cultures (BCs) (Wisplinghoff et al., 2004). They are important agents of bloodstream infections, but generally contaminate BCs as a result of permanent members of the skin flora (Rupp and Archer, 1994). For this reason, it is difficult to understand whether presence of CoNS represents true bacteremia or contamination (Centers for Disease Control and Prevention (CDC)). Many clinical and microbiological guidelines have been published to distinguish true bacteremia from contamination. One of them is the Laboratory-Confirmed Bloodstream Infection (LCBI) criteria provide further explanation and correct application in defining bloodstream infections. (Chandrasekar and Brown, 1994). However, using routine laboratory methods such as determination of species, phenotypic characteristics, and antibiotic resistance patterns are not likely to be helpful to distinguish true CoNS bacteremia from contamination (Baddour et al., 1990). Identification of the species of isolates does not determine strain relatedness within the same species (MacGregor and Beaty, 1972). Also, phenotypic characteristics do not show variations among several genetically related isolates (Souto et al., 1991). These variables have no high positive predictive values, and they are useful only when 2 or more BCs are positive in one series (García et al., 2004). Despite the use of multiple BC positivity as a good indicator for true bacteremia, studies in recent years have shown that 34% of patients with nosocomial bacteremia had only one BC positivity (MacGregor and Beaty, 1972). Using clinical assessment, Kirchhoff and Sheagren found that 52.9% of patients with CoNS bacteremia in multiple BCs did not have corresponding clinical signs to suggest true bacteremia (Kirchhoff and Sheagren, 1985).

There is still no single criterion with sufficient specificity to differentiate true CoNS bacteremia from contamination. The aim of this study to evaluate the efficiency of LCBI criteria in CoNS bacteremia and the clinical use of these criteria to distinguish CoNS bacteremia from contamination, and compare the results with each other.

2. Materials and methods

2.1. Study population

A prospective study was conducted at Istanbul University Cerrahpasa Medical School, a 1,300-bed tertiary care teaching hospital, between November 2009 and April 2010 in Turkey. The study was approved by the ethics committees of Cerrahpasa Medical Faculty. We included 162 adult patients (≥18 years old) who were treated by the various services of our hospital and had CoNS-positive BCs collected from intravenous catheters.

2.2. Data collection

The demographic and clinical data of the patients were collected, including invasive procedures, antimicrobial therapy, underlying

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diseases, relevant symptoms and signs related to bacteremia, vital signs, repeated BCs, duration of hospitalization, and clinical outcomes.

2.3. Microbiologic methods

BCs were taken according to the routine procedures (Thomson, 2007; Wilson et al., 2007). The routine BC set included an aerobic and anaerobic bottle (Bactec Plus aerobic-anaerobic/F; Becton Dickinson, United States) was transported to the microbiology laboratory and incubated until flagged as positive or for 5 days in an automated BC system (Bactec 9240; Becton Dickinson, United States). Time to positive culture detection was recorded for each patient. Blood from positive bottles was Gram stained and subcultured by using standard conventional microbiological methods (Mahon et al., 2011). Susceptibility of the isolates to antibiotics was determined by disc diffusion test according to the Clinical Laboratory Standard Institute guidelines (Bauer et al., 1966; Clinical and Laboratory Standards Institute (CLSI), 2010). Determination of CoNS species was performed with the BBL™ Crystal™ Gram-Positive ID Kit (Becton Dickinson, United States) according to the manufacturer's instructions in the study. The same CoNS strains have already been identified by microbiological conventional methods in routine clinical laboratory (Mahon et al., 2011). So, the CoNS isolates have been identified twice in different ways with the same results. The strains of CoNS were stored at 70 °C for RAPD-PCR (random amplification of polymorphic DNA-polymerase chain reaction) analysis.

2.4. Molecular methods

The isolates of CoNS from the patients with multiple positivity BCs were classified into the distinct subtypes by using RAPD-PCR analysis. RAPD was performed essentially as described previously. Genomic DNA was purified from CoNS isolates with the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. ERIC-2 primer (5′-AAG TAA GTG ACT GGG GTG AGC-3′) was used for amplification and performed at PTC-200 Peltier Thermal Cycler (MJ Research, MA, United States). After amplification, PCR products were analyzed by horizontal agarose gel electrophoresis. The isolates were described and compared by using RAPD-PCR analysis (Casey et al., 2006; Bingen et al., 1995).

2.5. Study design and definitions

Our study analysis consisted of 2 parts. In part 1, the patients were evaluated for CoNS bacteremia by clinicians. We recorded the results of clinical assessments for each patient. Then, each BC was evaluated according to the LCBI criteria of Centers for Disease Control and Prevention (CDC). LCBI contains the following criteria; patients should have at least one of the following signs or symptoms: fever (>38 °C), chills, or hypotension; microorganism cultured from the blood should not be related to an infection at another site; if the microorganism was bacterial skin flora, it must be identified from 2 or more BCs received under at least 2 different conditions; for skin flora bacteria, at least one of the BC sets must be taken via 2 different conditions and must report the same microorganism; the identification of the species must confirm the same microorganism in the 2 different conditions. Only genus and species identification should be utilized to determine the sameness of organisms (matching organisms). No additional comparative methods should be used (morphology or antibiograms) (Centers for Disease Control and Prevention (CDC)).

In part 2, outcome comparisons between the patients according to the LCBI criteria and the clinical evaluations were performed to demonstrate the difference of approaches between the CoNS bacteremia and contamination.

2.6. Statistical analysis

Age, sex, fever (>38 °C), the presence of risk factors (intravenous catheter, surgery, and TPN [total parenteral nutrition]), duration of hospitalization (day), and time to positive culture detection (hour) were screened as variable predictors by Welch *t* test. *P* values of less than 0.05 were regarded as significant.

3. Results

During the study period, a total of 232 CoNS were isolated from 197 CoNS-positive BC bottles of 162 adult patients. The median age of the study patients was 56 years, and 84 (52%) of the patients were male and 78 (48%) were female. Of the 162 patients, 21.6% (35) had 2 or more CoNS-positive BCs and 78.4% (127) had a single CoNS-positive BC. Among the 35 patients with 2 or more CoNS-positive BCs, 24 (68.5%) and 11 (31.5%) had the same and different CoNS species, respectively. Based on the LCBI criteria, 24 (15%) patients with the same species of CoNS in multiple BCs were classified as having true bacteremia, and 138 (85%) patients with a single CoNS-positive BC or with different CoNS species in multiple positive BCs were classified as contaminated. However, the RAPD-PCR analysis showed that 9 of the 24 patients had different CoNS genotypes. In the clinical assessments, only 12 of the 24 CoNS bacteremia patients and 8 patients with a single CoNS-positive BC had the clinical signs that suggest true bacteremia. Using this classification, 8 (6.2%) of the 127 patients with a single CoNS-positive BC and 12 (34.2%) of the 35 patients with 2 or more CoNS-positive BCs had true bacteremia.

S. epidermidis was the most frequently isolated species for both the true bacteremia (70%) and contaminated patients (64%). Eighty-one CoNS isolates from the 35 patients with 2 or more CoNS-positive BCs were performed by RAPD-PCR (Fig.). Of the 35 patients, 15 (43%) and 20 (57%) had the same and different CoNS genotypes, respectively. Fourteen of the 24 patients with the same species of CoNS also had identical antibiotic sensitivity patterns, but 8 of them had different CoNS genotypes. Clinical characteristic and laboratory outcomes of the patients with CoNS bacteremia are shown in Table 1.

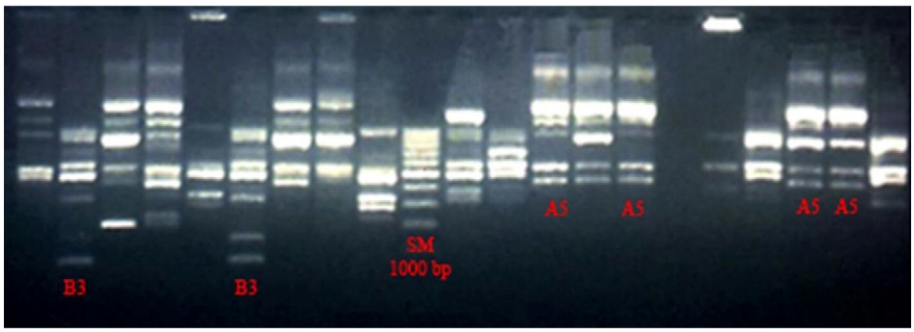

Figure. DNA fingerprinting analysis of CoNS isolates by RAPD-PCR. (A) *S. epidermidis*, (B) *S. haemolyticus*. A5: an epidemic strain, the subtype of *S. epidermidis*.