Molecular Typing of Blood Stream Infections Caused by Staphylococcus epidermidis

Using DiversiLab System and PFGE


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BACKGROUND

Staphylococcus epidermidis is a coagulase-negative staphylococci that colonizes the skin and mucous membranes of the human body, representing the majority of the normal bacterial flora in this habitat. Typically classified as only a contaminant when seen in the clinical setting, S. epidermidis is now considered a significant nosocomial opportunistic pathogen causing various infections to immunocompromised patients or patients with indwelling catheters and implanted devices. The success of S. epidermidis as a pathogen is largely due to the formation of extracellular material known as biofilm that surrounds the multi-layered cell clusters and allows them to adhere and remain on surfaces. Treatment of S. epidermidis is difficult as 80% of all strains are resistant to antibiotics and other common antibiotics. Nosocomial outbreaks have a tremendous impact on the morbidity, mortality, and cost associated with hospital-acquired infections. An estimated $5 billion per year in added treatment costs is spent as a result of infections that patients acquire while under hospital care for another illness. Several molecular typing methods are used to help determine possible sources of hospital-acquired infections including pulsed-field gel electrophoresis (PFGE) and random amplified polymorphic DNA (RAPD). A commercially available method, DiversiLab System, based on repetitive sequence-based PCR (rep-PCR), has proven useful in strain typing bacteria and fungi. This study reports and compares the performance of the DiversiLab System to standard PFGE analysis using clinical isolates collected from blood stream infections from three geographical regions outside the United States.

METHOD

Thirty-seven clinical S. epidermidis isolates were collected from three different healthcare centers. Samples were collected as part of an antibiotic resistance surveillance study (SENTRY). The isolates were tested using the DiversiLab System (Fig. 1a, Bacterial Barcodes, Inc., Athens, Georgia). The isolates were cultured on tryptic soy agar with 5% sheep blood and incubated at 35°C for 48 hours. The DNA from each culture was extracted using the UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Fig. 1a). The DiversiLab System repetitive DNA Fingerprinting Kit (Bacterial Barcodes, Inc.) was used for rep-PCR amplification of non-coding intergenic repetitive elements in the genomic DNA. The amplifications (Fig. 2a) were analyzed using the DiversiLab System which includes fragment separation using microfluidics lab-on-chip technology (Fig. 1b). Analysis of the PCR patterns was completed using manual interpretation and defined using Tenover criteria: indistinguishable—no band differences, closely related—two to three band differences, and different—three or more band differences. PFGE was carried out using the CHEF-DR™ (Bac-Rad Laboratories) electrophoresis system following a previously described method. Analysis of the PFGE patterns was completed using manual interpretation and defined using Tenover criteria: indistinguishable—no band differences, closely related—two to three band differences, and different—four or more band differences. PFGE was carried out using the CHEF-DR™ (Bac-Rad Laboratories) electrophoresis system following a previously described method. Analysis of the PFGE patterns was completed using manual interpretation and defined using Tenover criteria: indistinguishable—no band differences, closely related—two to three band differences, and different—four or more band differences. 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RESULTS

Rep-PCR fingerprints and PFGE patterns were obtained from S. epidermidis isolates from three countries, including Canada, Spain, and Turkey. The rep-PCR dendrogram and PFGE images from 12 isolates collected in Canada are shown in Figure 2. The interpretations obtained from each technique are shown in Table 1.