An Exploratory Study of *Staphylococcus epidermidis* Isolates from Environment and Patient Blood Cultures from an Intensive Care Unit

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Abstract This exploratory study determined the epidemiological characteristics of *Staphylococcus epidermidis* from near touch sites and blood cultures in one Intensive Care Unit (ICU) between July and October 2009 in a large teaching hospital in Brazil. Five different touch sites around the environment of two different patients were sampled over four days and 24 blood cultures from 24 patients were obtained and analyzed. Isolates from near touch sites and blood cultures were compared by means of the repetitive extragenic palindromic sequence-based polymerase chain reaction (rep-PCR) and analyzed by the unweighted pair-group method with arithmetic mean (UPGMA) (BioNumerics, 6.0). Results found that ciprofloxacin-resistant *Staphylococcus epidermidis* on stethoscopes, bed rails ($P < .004$), and patient blood cultures indicated similarity (60–65%; IC: 85%). The similarities among near touch sites and patient isolates are unsurprising due to the commensal nature of *Staphylococcus epidermidis*, however it does emphasize the need for further research into environmental contamination and cleaning.

Keywords *Staphylococcus epidermidis*; near touch sites; intensive care units; environmental contamination; environmental cleaning

1. Background

As healthcare-associated infections (HCAIs) are one of the top five causes of death, the prevention of HCAIs is recognized as a critical factor for safe healthcare [27]. Transient microorganisms can be transmitted by direct contact and inanimate objects by healthcare workers and patients and visitors [23,25]. One such microorganism is *Staphylococcus epidermidis*, a coagulase-negative staphylococcus which colonizes human skin and mucosal surfaces. It used to be considered as nonpathogenic in that it generally causes little or no damage to the host. However, increasingly in specific groups of patients, it is being recognized as being responsible for bacteremias associated with intravenous devices, such as central venous catheters [33] and peripheral intravenous cannula [24]. As most cases of *Staphylococcus epidermidis* bacteremia are associated with infected intravascular devices [2,15] for a minority of patients, it has the potential to cause harm due to the extracellular slime that *Staphylococcus epidermidis* produces, which adheres to medical devices, then multiplies and penetrates tissue or invades cells [20].

It is unsurprising then that the patients who are most susceptible to infections caused by *Staphylococcus epidermidis* are those with severe diseases, mainly those with a compromised defence system due to applied therapies, surgeries, or the use of invasive devices, most generally in ICUs [28]. The extent to which such susceptible patients’ environments act as a reservoir for infections is contradictory with some authors [3,8,10,16] suggesting that transmission of HCAIs by this route is plausible. Some studies described that the contribution of the environment is negligible [7,9,12,14,18]. Our study’s objective was to determine the extent of *Staphylococcus epidermidis* environmental contamination and subsequent risk of infection.

2. Methods

2.1. Setting

The study was carried out in an adult ICU located in a general and public university hospital in Belo Horizonte, Minas Gerais, Brazil, between July and October 2009. This study was approved by the Ethics Committee (COEP), ETIC report number 113/09.

2.2. Study design

Near touch sites included bed rails, cardiac monitor, mechanical fan, bedside table, and stethoscope from patients in isolation rooms within a general ICU. These near touch sites were swabbed using a sterile cotton tipped swab (Plastic nail - J.P) moistened with sterile saline solution which was moved across the 1 cm² delimited on the surface
of the near touch sites. Routine blood cultures from 24 adult ICU patients were obtained from hospital laboratory according to unit protocol.

The near touch site samples were distributed within Brain Heart Infusion (BHI) MacConkey and Sabouraud Agar solution culture mediums. All mediums were incubated at 37 °C for 48 h. Colonies with different morphologies were isolated. The isolates were submitted to Gram stain, as well as to biochemical and physiological tests (API Identification Kit–20 Staph, BioMérieux, Marcy L’Étoile, France). After identification by the API Kit, an antibiogram was carried out with the *Staphylococcus epidermidis* isolates (Bauer-Kirby method) and with the antimicrobials most used in the ICU: vancomycin (30 µg) and ciprofloxacin (5 µg). The bacterial isolates were separated into the categories of sensitive, intermediate, and resistant according to the criteria established by Clinical and Laboratory Standards Institute (CLSI) 2009. Isolates with a resistance profile were selected for analysis by repetitive extragenic palindromic sequence-based polymerase chain reaction (rep-PCR) [29].

The total DNA of *Staphylococcus epidermidis* isolated from inanimate surfaces and patients blood culture was extracted. For the rep-PCR, the primer (GTG)5 and Box (0.3 µM) were used. To evaluate PCR results, samples were subjected to a 2% agarose gel electrophoresis (Invitrogen, Carlsbad, CA, USA) for two hours at 70 voltz, and stained by ethidium bromide and photographed under ultraviolet light. For analysis of DNA bands, a 1 Kb Plus DNA Ladder (molecular marker, 75–20,00 bp, ready-to-use; Fermentas, Burlington, ON, Canada) was used.

The profiles generated by the rep-PCR were analyzed by using the BioNumerics version 6.0 program (Applied Maths, St. Martens-Latem, Belgium). The comparison between the profile pairs of the BOX-PCR and the (GTG)5 was calculated using the Pearson correlation coefficient. The analysis for grouping similar values was carried out by using the UPGMA algorithm. The data were analyzed by the Kruskal-Wallis test in the SPSS version 13.0 statistics program. This study was approved by the Ethics Committee, number 113/09.

### 3. Results

#### 3.1. Environmental contamination with *Staphylococcus epidermidis*

A total of 96 near patient touch site samples were obtained. Of these, 50 (52.1%) presented bacterial growth. Of the 50 positive samples, 51 bacterial isolates were obtained. Of these isolates, 3 (5.9%) were *Staphylococcus epidermidis* with an identification percentage of 93.1% of the species identified by the API Kit. Of these, 66.6% presented resistance to ciprofloxacin. All the isolates of *Staphylococcus epidermidis* from the ICU environment were sensitive to vancomycin. The total of 96 obtained samples were defined in regarding to several studies that used 1 to 52 different times of collection. In these studies, the total of analyzed samples ranged from 100 to 1,200 in periods of 2 to 22 of collection [1,16,29].

As regards the blood cultures of the ICU patients, 24 plates were obtained, in which 18 isolates were found. Of the isolates, eight were Gram negative (bacilli and two coccobacilli) and ten (55.5%) were *Staphylococcus epidermidis*. The profile of sensitivity towards antimicrobials was confirmed by the Bauer-Kirby method for ciprofloxacin and vancomycin. Of these isolates, seven were classified in the intermediate category concerning the response pattern to ciprofloxacin. In the resistance category concerning ciprofloxacin, 4 (40%) isolates from patient blood cultures were identified. By contrast, no isolates presented a profile of resistance to vancomycin.

#### 3.2. Distribution of *Staphylococcus epidermidis* in ICU

In the ICU environment, among another identified bacteria species the resistant isolates of *Staphylococcus epidermidis* were found on the bed rail and stethoscope (*P < .004*, Kruskal-wallis test) within the isolated hospital room (Figure 1).

The *Staphylococcus epidermidis* was found on the surfaces that have frequently contact with the patients, suggesting that proximity with colonized patient enhances the possibility of surfaces contamination.
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Figure 2: Grouping of *Staphylococcus epidermidis* according to the band profiles generated by GTG(5) (Belo Horizonte, 2010).

3.3. Isolates similarity of *Staphylococcus epidermidis*

Regarding the similarity, nine isolates of *Staphylococcus epidermidis* (3 from the environment and 6 from the blood culture) were compared by means of rep-PCR. As regards the BOX-PCR and GTG(5), the *Staphylococcus epidermidis* isolates taken from the stethoscope, from bed rail within the isolation room, and from the blood culture were grouped with a 60% (confidence interval (IC): 85%) similarity among the samples (Figure 2). The API Kit presented a 93.1% identification.

The similarity percentual among *Staphylococcus epidermidis* isolates from environment and blood culture samples was close to the 70%; a value proposed by Wayne et al. [31] for a single specie.

4. Discussion

One cohort study in a surgical intensive care unit [32] found that the frequency of cross transmission is especially high and in the ICU studied [16] it was 37.5% demonstrated environmental contamination with drug-resistant Gram positive bacteria was far greater than with Gram negative bacteria and concluded that there has been little evidence examining the environmental colonization of Gram negative pathogens. The few studies that have been undertaken [17, 26, 30] were further supported by Lemmen et al. study [16] which found that less than 5% of environmental contamination was positive.

However, more recently, there has been growing evidence [5, 6] that the environment and the issue of environmental cleaning and decontamination are important factors at minimizing HCAIs. Some authors [5, 6, 22] argue that environmental cleaning needs to be improved generally and specifically at hand-touch sites and Randle et al. [23] recommend that further research and policies need to concentrate on near touch patient surroundings. Hand touch sites which provide the highest risk to patients are those which are next to the patient, for example, bed rails, lockers, overbed tables, and door handles [22]. Shared equipment can act as a vehicle by which cross transmission occurs and can cause infections [19]. One systematic review [13] found that over half of the inanimate objects such as those previously listed were not microbiologically clean when swabbed and another study [21] suggests extensive contamination of MSSA and MRSA in the hospital environment. Poor environmental hygiene has been associated with transmission of pathogens and causing HCAIs [4, 11] and it is for these reasons that although we recognize that just because microorganisms are present in the environment it does not always mean that these cause HCAIs, but it does warrant further investigation.

5. Conclusions

The similarities among near touch sites and patient isolates are unsurprising due to the commensal nature of *Staphylococcus epidermidis* and there is no direct evidence that the bacteria found directly caused infection, however it does emphasize the need for further research into environmental contamination and cleaning.

Conflict of interest No conflict of interest has been declared by the author(s).
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