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Scientific Research

Comparing Resistant Microorganisms Isolated from Patients and Environment in an Intensive Care Unit

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Abstract

Background: Recently, the probable involvement of surfaces from the hospital environment as a disseminating source of resistant bacteria has been highlighted. The aim of the study was to compare resistant microorganisms isolated from inanimate surfaces, equipments and patient blood culture samples in an Intensive Care Unit from Belo Horizonte, Brazil. Methods: A cross-sectional study was performed from July to October 2009. Data sources were microbiologic samples from environment and patient blood culture. Duplicate samples were obtained by swabs from up to seven different touch sites around two different patients in four different days. Jointly with the environmental samples, bacterial isolates from an adult ICU patients' routine blood cultures were obtained from hospital laboratory. The samples were identified, tested for sensitivity and compared by rep-PCR test to verify similarity. Results: Difference among the averages of Colony Forming Units was found within the environment samples ($p < 0.004$). In the environment were identified antibiotic resistant microorganisms such as Vancomycin resistant *Enterococcus faecalis*, imipenem and ciprofloxacin *Pseudomonas aeruginosa* and multidrug-resistant *Acinetobacter baumannii*. Similarities (60% - 80%) were established among environmental and blood culture samples. Conclusion: The environmental sampling showed different averages of contamination of the surfaces and equipment. The similarity among the bacterial isolates of patients' blood cultures and environmental samples reinforces the hypothesis of the horizontal transference of pathogens.

Keywords

Cross Infection; Bacterial; Drug Resistance; Intensive Care Units; Environment; Contamination

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1. Introduction

The surfaces near colonized patients, or those touched frequently by health professionals and by those who move within the area, can become contaminated by antibiotic resistant bacteria, especially methicillin resistant *Staphylococcus aureus*-MRSA [1] [2] and vancomycin resistant *enterococcus*-VRE [3].

Recently, the probable involvement of surfaces and equipment from the hospital environment as a disseminating source of pathogens, including resistant bacteria, has been highlighted [4]. There are no meaningful standards for permissible levels of microbial contamination of inanimate surfaces in hospital environment, but an increased microbial load on surfaces may imply the possibility of finding a pathogen [5].

The spread of pathogens in the hospital environment may be favored by some aspects such as the quantities of equipment and surfaces and patients status. One such example is the intensive care units (ICUs) with patients under intensive care [2] [3] [6].

Therefore, within this context, it is pertinent to question if there is in fact a relationship between bacterial isolates from patient's clinical samples, such as the routine blood culture, and the possible contamination of the inanimate surfaces, equipment and solutions of an ICU, considering the microorganisms of greater prevalence within the literature and of epidemiological importance within the institution of study (*Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterococcus* spp.).

The aim of this study was to compare resistant microorganisms isolated from inanimate surfaces, equipments and patient blood culture samples in an Intensive Care Unit (ICU) from Belo Horizonte, Brazil.

2. Materials and Methods

2.1. Study Design

This was a cross-sectional study of an adult ICU from a University hospital in Belo Horizonte, Brazil, from July to October 2009. Duplicate samples were obtained by swabs from up to seven different touch sites around two different patients in four different days. The surfaces sampled were from the command buttons of the cardiac function monitor, from the tracheal tube connected to the mechanical ventilator, the bed rail, the internal rims of the faucet, the top of the bedside table, the diaphragm of the stethoscope, and the upper borders of the sink, within a patient unit of an isolation unit or rooms. The sampling area for each surface was 1 cm^2. The surfaces for sampling were defined according to those described in other studies as contaminated by epidemiological important bacteria [1]-[5] [7]-[11].

Jointly with the environmental samples, bacterial isolates from an adult ICU patients' routine blood cultures were obtained from hospital laboratory according to the unit protocol.

2.2. Microbiological and Molecular Tests

The samples from the ICU environment were distributed within culture mediums of Brain Heart Infusion (BHI), MacConkey, and Sabouraud Agar solutions and incubated at 37˚C for 48 hours. The semiquantitative count of microbial growing was done for each medium. Colonies were enumerated and used to calculate the Colony Forming Units (CFU)/ml. Colonies with different morphologies were isolated. The isolates were submitted to Gram coloration as well as to biochemical and physiological tests (API identification Kits from BioMérieux, Lyon, Marcy-L'Etoile). After the identification of the API Kit, an antibiogram was performed with the bacterial isolates (Bauer-Kirby method) and with the more commonly used antimicrobials within the ICU—vancomycin, imipenem, ciprofloxacin, and ceftriaxone. In the case of *Staphylococcus aureus* and *Enterococcus faecalis*, the test of susceptibility to vancomycin was carried out by applying the technique of diluting the antimicrobial in Agar solution [12]. *Staphylococcus aureus*—ATCC 29213 was used for control of the test of susceptibility. The isolates with a resistance profile were selected for analysis using the repetitive extragenic palindromic sequence—Polymerase chain reaction (rep-PCR).

The total DNA was extracted. For the rep-PCR, the primers (GTG) 5 and Box (0.3 µM) were used. To view the PCR results, the samples were run through a 2.0% agarose gel (Invitrogen-Carlsbad, CA) for two hours at 70 voltz, colored by ethidium bromide and photographed in ultra-violet light. For this, a 1 Kb Plus DNA Ladder, ready-to-use, 75 - 20.00 bp (Fermentas-Burlington, Ontario, Canada) molecular marker was used.

2.3. Data Analyses

The profiles generated by the rep-PCR were analyzed 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 were calculated using the Pearson correlation coefficient. The analyses for grouping similar values were carried out using the Unweighted pair-group (UPGMA) algorithm. The data were analyzed by the Kruskal-Wallis test within the SPSS version 13.0 statistics program. This study was approved by the Ethics Committee, number 113/09.

3. Results

The Kruskal-Wallis test identified an important difference among the averages ($P < 0.004$) of Colony Forming Units-CFU/ml found within the samples obtained by swabs of the cardiac function monitor, the mechanical ventilator, the stethoscope, the bed rail, the faucet, the bedside table, and the sink (**Table 1**).

The identification of bacterial isolates by API kit (ranged from 77.5% to 99.3%) and antibiogram performed by Bauer-Kirby method showed epidemiological important microorganisms within the studied ICU, the following were found in the patient isolation units: imipenem, ciprofloxacin, and ceftriaxone resistant *Acinetobacter baumannii*; ciprofloxacin resistant *Staphylococcus aureus*; and vancomycin and ciprofloxacin resistant *Enterococcus faecalis* (**Figure 1**).

From the blood cultures of patients, were isolated four Gram negative bacilli, resistant to ceftriaxone and/or ciprofloxacin, tree *Staphylococcus epidermidis* resistant to ciprofloxacin, and four were classified as intermediary concerning their pattern of response toward ceftriaxone and ciprofloxacin.

The similarity among environmental and patient's blood culture samples amplified by the GTG (5) and BOX primers ranged from 60% to 80% (Confidence Interval-IC: 85%).

4. Discussion

The environmental sampling by swabs showed different averages of contamination of the surfaces and equipment that may be related to: the proximity of the equipment or surface to the patient, the frequency with which professionals and people who visit the sector touch the objects, decontamination, type of material, among other factors [4] [13].

During concurrent cleaning, the environment decontamination of the studied adult ICU is performed according to the routine protocol, including the daily decontamination of the surfaces with 70% ethyl alcohol. During terminal cleaning, after patient discharge, transference, or death, the entire unit, including the floors and walls, are washed, followed by the application of sodium hypochlorite (10.000 ppm) for non-metallic surfaces and floors.

The detection of the resistant bacteria on surfaces and equipment is most likely related to the possibility of recontamination, several hours after the hygienization of the environment, or even due to the possible of some microorganisms persist on surfaces after cleaning, in addition to the high frequency of manipulation and touch, as they refer to locations that demand repetitive re-evaluation of the parameters of the patient in his/her clinical condition [9] [14].

The presence of multidrug—resistant *Acinetobacter baumannii* on room surfaces, even after four rounds of routine terminal cleaning and disinfection of rooms newly vacated by patient carrying this microorganism, was found by Manian *et al*. [15]. The terminal cleaning and disinfection of rooms included "wiping of visibly soiled

Table 1. Average of Colony Forming Units/ml from equipments and surfaces samples from an Intensive Care Unit—Belo Horizonte, 2010.

Equipments	Colony Forming Units	Surfaces	Colony Forming Units
Cardiac Monitor	92.5 (9.25 × 10 CFU/ml)	Bed rail	305 (3.05 × 10^2 CFU/ml)
Mechanical Ventilator	313.25 (3.13 × 10^2 CFU/ml)	Faucet	2098.3 (2.09 × 10^3 CFU/ml)
Stethoscopes	740 (7.4 × 10^2 CFU/ml)	Bedside table	80 (8.0 × 10 CFU/ml)

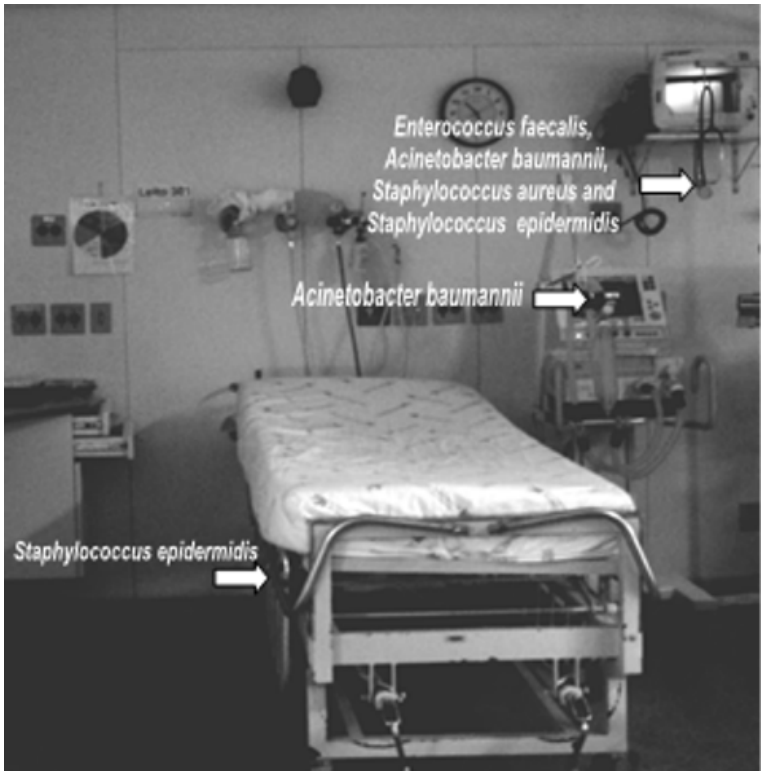

Figure 1. Distribution of resistant bacteria on surfaces and equipment in an isolated room of an ICU—Belo Horizonte, 2010.

surfaces with a quaternary ammonium compound followed by disinfection with 0.525% sodium hypochlorite solution (1:10 dilution)" [15].

The contamination of surfaces with resistant microorganisms may increase its spread among patients and hospital environment. Hayden *et al*. [3] found that 52% of healthcare workers had the hands or gloves contaminated with VRE after touching the environment in a room occupied by a patient carrying this microorganism [3].

In studies on the environment's participation in the dissemination of pathogens, the contamination of surfaces near the patient or those touched many times by health professional, and fomites could be observed. On surfaces of the bed, *Acinetobacter baumannii*, VRE, and MRSA were the most common, with the last being the most frequent. In addition, these were found on door-knobs, chairs, toilet seats, tables, computer keyboards, and diaphragms of stethoscopes [1] [2] [6] [9] [16] [17]. On the faucets, *Pseudomonas aeruginosa* was the most common, especially in humid locations [8] [18] [19].

In this study according to the grouping of profiles generated by the rep-PCR, with primers GTG [5] and BOX of the bacterial isolates, no significant diversity among the samples from the ICU environment and patient blood cultures in the sector could be observed, in accordance with the similarly percentage of 70% proposed for species level [20].

5. Conclusions

In conclusion, it was possible to verify similarity among some isolates of resistant bacteria recovered from equipment and inanimate surfaces and patient blood culture. Such a similarity among the bacterial isolates of patients' blood cultures and environmental samples reinforces the hypothesis of the horizontal transference of pathogens.

The contamination of the equipment and surfaces by resistant bacteria reinforces the need to pay closer attention to the disinfectants used due to the possibility of favoring the dissemination of different pathogens. Most notably is the importance of the reinforcement of the control, reduction and prevention measures applied against the dissemination of the resistant microorganisms. These aspects are not analyzed in the present study, but they

are of great relevance within this context.

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