Resistance of bacteria isolated from equipment in an intensive care unit

Resistência de bactérias isoladas em equipamentos em unidade de terapia intensiva

Igor Vasconcelos Rocha¹
Patrick de Mélo Ferraz¹
Thaísa Gabriela Silva de Farias¹
Sibele Ribeiro de Oliveira¹

Keywords

Nursing service, hospital; Equipment contamination; Drug resistance, microbial; Cross infection/prevention & control; Intensive care units

Descritores

Serviço hospitalar de enfermagem; Contaminação de equipamentos; Resistência microbiana a medicamentos; Infecção hospitalar/ prevenção & controle; Unidades de terapia intensiva

Submitted

March 8, 2015

Accepted

March 31, 2015

Corresponding author

Igor Vasconcelos Rocha Portugal Avenue, 584, Caruaru, PE, Brazil. Zip Code: 55016-430 igorocha17@gmail.com

DOI

http://dx.doi.org/10.1590/1982-0194201500073

Abstract

Objective: Evaluate drug resistance of bacteria isolated from equipment placed close to patients in an Intensive Care Unit of a hospital in Caruaru/Pernambuco, Brazil.

Methods: This is a cross-sectional study. The samples were collected with swabs moistened with Trypticase Soy Broth, which were then cultured in sheep blood agar and MacConkey agar. The phenotypic identification performed was based on the morphology of the strains and biochemical results. The drugs resistance analysis was based on Kirby-Bauer's Disk Diffusion protocol.

Results: A rate of 94.4% of the analyzed equipment was contaminated. The most frequently isolated microorganisms were: *Acinetobacter* sp., *Staphylococcus aureus* and *Pseudomonas* sp. Just about 75% of *Acinetobacter* sp. was resistant to piperacillin associated to tazobactam, meropenem and levofloxacin. Similarly, 36.3% of *S. aureus* showed resistance to oxacillin and 10% of *Pseudomonas* sp. was resistant to the drugs tested.

Conclusion: Most of the microorganisms presented high levels of resistance to the drugs.

Resumo

Objetivo: Avaliar a resistência microbiana a medicamentos de bactérias isoladas de equipamentos próximos aos pacientes da Unidade de Terapia Intensiva.

Métodos: Trata-se de um estudo transversal. As amostras foram coletadas com *swabs* umedecidos em *Trypticase Soy Broth*, semeados posteriormente em Ágar Sangue de Carneiro e *MacConkey*. A identificação fenotípica ocorreu com base na morfologia das cepas e resultados bioquímicos. A análise da resistência aos medicamentos foi baseada no método de disco-difusão de *Kirby-Bauer*.

Resultados: Apresentaram-se contaminados 94,4% dos equipamentos analisados. Os microrganismos isolados mais frequentes foram: *Acinetobacter* sp., *Staphylococcus aureus* e *Pseudomonas* sp. Cerca de 75% de *Acinetobacter* sp. apresentaram resistência à piperacilina associada a tazobactam, meropenem e levofloxacina. Da mesma forma, 36,3% de *S. aureus* apresentaram-se resistentes à oxacilina e 10% dos isolados de *Pseudomonas* sp. foram resistentes aos medicamentos testados.

Conclusão: A maioria dos isolados apresentaram elevadas taxas de resistência microbiana aos medicamentos.

¹Associação Caruaruense de Ensino Superior e Técnico, Caruaru, PE, Brazil. **Conflicts of interest:** there are no conflicts of interest to declare.

Introduction

Healthcare-associated Infections (HAI) are responsible for thousands of deaths every year around the world. In Brazil, this problem increases in terms of numbers as well as complexity, causing economic and social disruption with high levels of morbidity and mortality. (1,2)

In Intensive Care Units (ICU), the contamination of equipment by bacteria is common, turning them into reservoirs of these microorganisms, enabling the colonization and cross infection of patients, complicating prognosis and favoring HAI outbreaks, mostly by microorganisms multiresistant to antibiotics commonly applied in therapeutics, which implies severe limitations to the treatment of hospital infections, posing a great threat to public health. (4)

Different organisms are related to contaminations in hospital environments and HAI processes, (5) but the main pathogens include oxacillin-resistant *Staphylococcus aureus* (ORSA), vancomycin-resistant *Enterococcus* sp. (VRE) and, more recently, extended-spectrum beta-lactamases (ESBL) and carbapenem-resistant *Acinetobacter baumannii*. (6-9)

Bacterial resistance is natural and unavoidable, (3) but the frequent and undistinguished use of antimicrobials (mainly broad-spectrum drugs) are crucial factors for the development and acceleration of this process. (3,10)

Given these facts, the purpose of this study was to isolate and determine the drug resistance profile of bacteria isolated from ICU equipment in a hospital in Caruaru-PE.

Methods

This descriptive and cross-sectional study was developed in the Intensive Care Unit of a hospital located in Caruaru, in the Northeast of Brazil, from January to December 2013.

Convenience sampling was applied, in which 54 pieces of equipment (right and left side rails and height adjustment buttons from the beds, infusion pump buttons, individual light switches and cardiac

monitor shelves) distributed among the nine beds present in the general ICU (Figure 1) were selected for collection. The inclusion criterion was samples from surfaces whose beds were occupied by their respective patients.



Figure 1. Equipment collected (arrows) in each bed

The collected data were typed, validated and processed in the software Excel 2010 (Microsoft Office*). Descriptive analysis was applied to obtain the percentage of samples.

The samples were collected six hours after the last time the hospital beds had been cleaned (corresponding to two hours after the end of the visiting period), so as not to interfere in the routine activities on site. Sterile swabs were used moistened with Trypticase Soy Broth (TSB) medium. Immediately after the collection, when the swabs were spun around their axis over the previously selected equipment, they were again stored in the medium and incubated at 36 ± 0.5 °C during 24 hours.

After the growth in TSB, the samples were seeded in sheep blood agar and MacConkey agar and also incubated at 36 ± 0.5 °C during 24 hours.

Gram staining was performed, followed by the identification of the genera and/or species of the bacteria, according to macro and microscopic characteristics of the colonies and biochemical test results. For the identification of bacteria from the family Enterobacteriaceae, the carbohydrate fermentation test was used in Triple Sugar Iron (TSI), as well as biochemical tests using the Sulfide Indole Motility (SIM), Simmons' citrate and Christensen's Urea Agar growth mediums. Tests based on Oxidase and Polymyxin B were used for the identification of glucose-non-fermenting Gram-negative bacteria. The identification of Staphylococcus sp. was performed through catalase, DNase and Novobiocin tests. Streptococcus sp. were identified through the characteristics of hemolytic activity, the use of Bile esculin agar, Brain Heart infusion (BHI) + NaCl 6.5% and optochin tests.

The drugs resistance analysis was based on Kirby-Bauer's Disk Diffusion protocol in Müeller-Hinton agar, as proposed by the Clinical and Laboratory Standard Institute (CLSI) 2013. (11)

Results

It was observed that 94.4% of the analyzed equipment was contaminated by one or more bacterial species. The most numerous isolated bacteria were Acinetobacter sp., Staphylococcus aureus, Coagulase-negative Staphylococci (CoNS), Staphylococcus saprophyticus, Enterococcus sp., Klebsiella pneumoniae and Streptococcus viridans, as presented in table 1. Gram-positive bacilli were found in 33.3% of the beds.

Among the *Acinetobacter sp.* isolated, 75% were resistant to imipenem, levofloxacin and piperacillin associated with tazobactam. 37.5% of the isolated microorganisms from this genus were resistant to ticarcillin, 31.2% to amikacin, 18.7% to ciprofloxacin, tetracycline and ceftazidime and 12.5% to gentamicin. 6.25% of the isolated microorganisms were found to have an intermediate level of resistance to ceftazidime and 12.5% to tobramycin.

With regard to the isolated *Staphylococcus aureus*, 72.7% were resistant to erythromycin, 63.6% to penicillin, 54.5% to clindamycin and ciprofloxacin and 18.8% to gentamicin. None of the isolated *Staphylococcus aureus* turned out to be resistant to cefoxitin. 9% of them had an intermediate level of resistance to oxacillin, clindamycin, erythromycin and ciprofloxacin. 36.3% were resistant to oxacillin (ORSA).

Among the Coagulase-negative *Staphylococci* strains, 71.4% and 54.1% were resistant to erythromycin and clindamycin, respectively. All these strains were susceptible to gentamicin, with 14.2% of them having the intermediate resistance phenotype to clindamycin. 42.8% were resistant to penicillin and 14.2% to tetracycline and cefoxitin.

The biggest number of cases of resistance to oxacillin is this study occurred in the isolated strains of *Staphylococcus saprophyticus*, which reached resistance levels in 85.7% of the samples, followed by 71.4% of resistance to erythromycin and clindamycin, 42.8% resistant to ciprofloxacin, 42.6% to cefoxitin, 28.5% to tetracycline and 14.2% to penicillin.

As regards the mechanism of inducible resistance to clindamycin, 12% of the *Staphylococcus*

Tabela 1. Distribution of bacteria isolated on equipment

Microorganism	S1	S2	S3	S4	S5	S6	Total
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Acinetobacter sp.	03(18.7)	04(25)	03(18.7)	01(6.25)	01(6.25)	04(25)	16(28.57)
S. aureus	04(36.3)	02(18.1)	01(9)	01(9)	02(18.1)	01(9)	11(19.64)
Pseudomonas sp.	01(10)	01(10)	02(20)	01(10)	01(10)	04(40)	10(17.85)
S. coagulase negativa	0(0)	0(0)	01 (14.2)	03(42.8)	02(28.5)	01(14.2)	07(12.5)
S. saprophyticus	0(0)	02(28.5)	03(42.8)	0(0)	01(14.2)	01(14.2)	07(12.5)
Enterococcus sp.	0(0)	0(0)	0(0)	01(33.3)	01(33.3)	01(33.3)	03(5.35)
Klebsiella pneumoniae	0(0)	0(0)	0(0)	0(0)	0(0)	01(100)	01(1.78)
S. viridans	0(0)	0(0)	0(0)	0(0)	0(0)	01(100)	01(1.78)
Total isolated	08(14.2)	09(16)	10(17.8)	07(12.5)	08(14.2)	14(25)	56(100)

S1 - right rail; S2 - left rail; S3 - height adjustment buttons from the beds; S4 - infusion pump buttons; S5 - individual illumination switches; S6 - cardiac monitors' shelf; n - number of isolated bacteria; % - percentage of isolated bacteria

sp. had the positive phenotype. This was detected through the disk approximation test with erythromycin and clindamycin (Figure 2), in which 66.6% corresponded to Coagulase-negative *S.* and 33.3% to *S. aureus*.

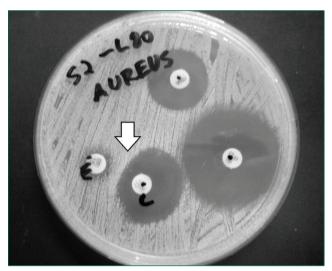


Figure 2. Inducible clindamycin resistance phenotype in *S. aureus.* E - erythromycin; C - clindamycin

Only 5.35% of the isolated strains corresponded to *Enterococcus* sp., 33.3% of which had intermediate levels of resistance to penicillin and ampicillin. None of the isolated strains were resistant to vancomycin.

A rate of 17.85% of the isolated strains corresponded to *Pseudomonas* sp., none of which turned out to have a significant level of resistance to the tested antibiotics (gentamicin, levofloxacin, aztreonam, ceftazidime, tobramycin, amikacin, ciprofloxacin, meropenem, chloramphenicol, cefoxitin and ticarcillin + clavulanic acid), while 10% of them were resistant and 20% presented intermediate levels of resistance to piperacillin + tazobactam; and 10% also had intermediate levels of resistance to ticarcillin + clavulanic acid and aztreonam.

Only one specimen of bacteria from the *Enterobacteriaceae* family (*Klebsiella pneumoniae*) was isolated in the study performed. Resistance was detected to ciprofloxacin, tetracycline, ampicillin, chloramphenicol and gentamicin, intermediate resistance to piperacillin + tazobactam and susceptibility to meropenem and tobramycin, and a neg-

ative result was obtained for Extended-Spectrum Beta-Lactamase (ESBL) production.

Discussion

Part of the results reported in this study (be it the occurrence of genera and/or species of bacteria or their antimicrobial resistance profiles) corroborate what is described in the scientific literature, although comparisons sometimes tend to be inaccurate, since the sampling and microbial detection methods vary considerably among different studies. (2)

The high number of Acinetobacter sp. isolated may have been due to its high level of nutritional and metabolic versatility, which allows this genus to use a large variety of substrates as carbon sources, remaining active for days or weeks in hospital environments, (12,13) which highlights the importance of their detection in such places, as this microorganism is directly involved in various ICU-related clinical complications, (2) and also in mechanisms of acquired resistance to carbapenem antibiotics. (4) Despite the existence of studies^(2,4,6,13) on the genus Acinetobacter as an HAI agent from clinical samples, there is a lack of statistical data on the prevalence of this bacterium on hospital equipment. Besides that, the occurrence of *Acinetobacter* sp. with multiresistant profiles is also considerable, which shows the need for more complex studies to determine the behavior of these strains in this kind of environment.

As regards the resistance profile of *S. aureus* strains to oxacillin (ORSA), a low number of cases was detected compared to the number of Gram-positives, although the result turned out to be superior when compared to a similar study, in which only 11.8% of the isolated strains had this resistance profile. When the resistance of *S. aureus* strains to cefoxitin is analyzed, the results described in this study were similar to those of a study performed with biological materials, in which no strains with this resistance profile were reported. (16)

As for the Coagulase-negative *Staphylococci* strains, no studies on their resistance profile in ICU

equipment were found, but a study from 2006⁽¹⁷⁾ reported lower resistance levels to erythromycin and clindamycin in biological samples: 68.7% and 63% respectively.

The occurrence of *S. saprophyticus* described in this study was superior when compared to studies from Libya, performed in 2014, in which only 3.3% of the analyzed pieces of hospital equipment were contaminated with this bacterial species. (18) Although the authors did not publish data on the antimicrobial resistance profile of *S. saprophyticus*, the levels for the genus *Staphylococcus* corresponded to 71.4% and 38.1% for erythromycin and ciprofloxacin, respectively, (18) corroborating the importance of their detection.

Despite the small number of *Staphylococcus* sp. strains with positive phenotype to induced resistance to clindamycin, this finding ought to be considered with special attention since the environment in question is one in which the patients are immunocompromised. Thus, the small number of occurrences must not be underestimated, as these bacteria have a great potential to cause hospital infections. (2,3,5)

The low occurrence of vancomycin-resistant *Enterococcus* sp. strains differs from a study performed in the US using biological samples, (19) in which the estimated rate of resistance to this drug in ICU was of 17.7%. In Brazil, the first report of this phenotype dates back to 1998 and, in Latin America, the rise in the number of cases of this kind of resistance happened in countries like Chile, Uruguay and Argentina. (20,21)

The presence of *Pseudomonas* sp. strains as reported in this study corroborates what is reported in similar studies, (22-24) based on the analysis of equipment from different hospital environments, including ICU. In a hospital environment, the biggest sources of contamination from this microorganism are breathing equipment, hemodialysis systems, sinks and cleaning apparatus. The relevance of *Pseudomonas* sp. as a potential hospital pathogen depends on the bacterial species and is associated to its relative resistance to the drugs, as well as to its reduced susceptibility to the antiseptics and disinfectants

used in these environments. (25,26) The drugs resistance profile of *Pseudomonas* sp. here described corroborates previous studies performed with biological material, (27) in which the major part of the antibiotics tested on *Pseudomonas aeruginosa* turned out to be effective against most of the isolated strains. The presence of these strains with mutually similar drug resistance profiles suggests the dissemination of a clone in the hospital environment; a fact that is probably related to cross-contamination mechanisms, although more extensive studies would be needed to confirm its dispersion in the ICU environment.

With regard to the percentage of isolated strains belonging to the *Enterobacteriaceae* family, the results obtained in this study differ from what is reported in studies performed in equipment from hospital environments, (28) where 30.3% were found to be contaminated by strains of this family. On the other hand, the antimicrobial resistance analysis of these strains in this study corroborates what is reported in the scientific literature: the massive presence of resistance to aminoglycosides and third-generation cephalosporins. (7,29)

When ESBL-production is considered, the results described in this study differ from those reported by Weber *et al.*⁽³⁰⁾ and Judge *et al.*,⁽²⁸⁾ in which a total of 22.2% of *Enterobacteriaceae* strains isolated on equipment from ICUs beds were ESBL-producing.

Conclusion

Most of the isolated strains had high rates of antimicrobial resistance to the drugs, which poses a great threat to public health.

Collaborations

Rocha IV, Ferraz PM and Farias TGS declare that they contributed to the research planning, analysis and interpretation of data and actively participated in the writing of the paper and final approval of the version for publication. Oliveira SR collaborated in the research planning, writing of the paper, relevant critical review of the intellectual content and final approval of the version for publication.

References

- McKibben L, Horan T, Tokars JI, Fowler G, Cardo DM, Pearson ML, et al. Guidance on public reporting of healthcare-associated infections: Recommendations of the Healthcare Infection Control Practices Advisory Committee. Am J Infect Control. 2005; 33(4):217-26.
- Allegranzi B, Nejad SB, Combescure C, Graafmans W, Attar H, Donaldson L, et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. Lancet. 2011; 337(9761):228-41.
- Polin RA, Denson S, Brady MT; Committee on Fetus and Newborn; Committee on Infectious Diseases. Strategies for prevention of health care-associated infections in the NICU. Pediatrics. 2012; 129(4):e1085-93.
- Dettori M, Piana A, Deriu MG, Curto PL, Cossu A, Musumeci R, et al. Outbreak of multidrug-resistant *Acinetobacter baumannii* in an intensive care unit. New Microbiol. 2014; 37(2):185-91.
- Juayang AC, Reyes GB, Rama AJG, Gallega CT. Antibiotic resistance profiling of *Staphylococcus aureus* isolated from clinical specimens in a tertiary hospital from 2010 to 2012. Interdiscip Perspect Infect Dis. 2014; 2014:898457.
- Cieslinsk JM, Arend L, Tuon FF, Silva EP, Ekermann RG, Dalla-Costa LM, et al. Molecular epidemiology characterization of OXA-23 carbapenemase-producing *Acinetobacter baumannii* isolated from 8 Brazilian hospitals using repetitive sequence-based PCR. Diagn Microbiol Infect Dis. 2013; 77(4):337-40.
- Huh K, Kang CI, Kim J, Cho SY, Ha YE, Joo EJ, et al. Risk factors and treatment outcomes of bloodstream infection caused by extended-spectrum cephalosporin-resistant *Enterobacter* species in adults with cancer. Diagn Microbiol Infect Dis. 2014; 78(2):172-7.
- Tajeddin E, Rashidan M, Razaghi M, Javadi SSS, Sherafat SJ, Alebouyeh M, et al. The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections. J Infect Public Health. 2015; 446:1-11.
- Jeon JH, Hong MK, Lee JH, Lee JJ, Park KS, Karim AM, et al. Structure of ADC-68, a novel carbapenem-hydrolyzing class C extendedspectrum β-lactamase isolated from *Acinetobacter baumannii*. Acta Cryst. 2014; D70:2924-36.
- Buul LW, Steen JT, Veenhuizen RB, Achterberg WP, Schellevis FG, Essink RTGM, et al. Antibiotic use and resistance in long term care facilities. 2012; 13:568.e1-e13.
- Clinical and Laboratory Standards Institute. Performance Standards For Antimicrobial Susceptibility Testing, Twenty-Third Informational Supplement, M100-S21. Clin Lab Stand Inst. 2013; 32(3):1-184.
- Sand M, Stahl J, Waclawska I, Ziegler C, Averhoff B. Identification of an osmo-dependent and an osmo-independent choline transporter in *Acinetobacter baylyi*: implications in osmostress protection and metabolic adaptation. Environ Microbiol. 2014; 16(6):1490-502.
- Espinal P, Martí S, Vila J. Effect of biofilm formation on the survival of Acinetobacter baumannii on dry surfaces. J Hosp Infect. 2012; 80:56-60.

- Nseir S, Blazejewski C, Lubret R, Wallet F, Courcol R, Durocher A. Risk of acquiring multidrug-resistant Gram-negative *bacilli* from prior room occupants in the intensive care unit. Clin Microbiol Infect. 2011; 17(8):1201-8.
- 15. Faires MC, Pearl DL, Ciccotelli WA, Straus K, Zinken G, Berke O, et al. A prospective study to examine the epidemiology of methicillinresistant *Staphylococcus aureus* and *Clostridium difficile* contamination in the general environment of three community hospitals in southern Ontario, Canada. BMC Infect Dis. 2012; 12:290.
- Abbas I, Omer AF, Ali H. Detection of cefoxitin resistant *Staphylococcus aureus* in Khartoum Hospitals, Sudan, 2011. Am J Res Commun. 2015; 3(1):73-83.
- Alvarez C, Cortes J, Arango A, Correa C, Leal A, Grebo. [Anti-microbial resistance in Intensive Care Units in Bogotá, Colombia, 2001-2003].
 Rev Salud Pública. 2006; 8(1):86-101. Spanish.
- Ali MM, Albakush AM, Rzeg MM, Ghenghesh KS. Identification of multidrug-resistant bacteria and *Bacillus cereus* from healthcare workers and environmental surfaces in a hospital. Libyan J Med. 2014; 9:1-2.
- Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of *Enterococci*. Results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis. 2001; 32 Suppl 2:133-45.
- Bazet DC, Blanco J, Seija V, Palacio R. [Vancomycin-resistant enterococci: An emerging problem in Uruguay]. Rev Med Uruguay. 2005; 21:151-8. Spanish.
- Littvik AM, Lopez TN, González SE, Fernandéz CM, Pavan JV. Colonization with vancomycin-resistant *Enterococci* (VRE) in intensive care unit patients in Cordoba City, Argentina. Rev Argent Microbiol. 2006; 38(1):28-30.
- Abreu PM, Farias PG, Paiva GS, Almeida AM, Morais PV. Persistence of microbial communities including *Pseudomonas aeruginosa* in a hospital environment: a potential health hazard. BMC Microbiol. 2014; 14:118-28.
- Huang SS, Datta R, Plat R. Risk of acquiring antibiotic-resistant bacteria from prior room occupants. Arch Intern Med. 2006; 166(18):1945-51.
- Petignat C, Francioli P, Nahimana I, Wenger A, Bille J, Schaller MD, et al Exogenous sources of *Pseudomonas aeruginosa* in intensive care unit patients: implementation of infection control measures and follow-up with molecular typing. Infect Control Hosp Epidemiol. 2006; 27(9):953-7
- Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant *Pseudomonas aeruginosa*: Risk factors and clinical impact]. Antimicrob. Agents Chemother. 2006; 50(1):43-8.
- Moore JE, Rendall JC. Comparison of susceptibility of cystic-fibrosisrelated and non-cystic-fibrosis-related *Pseudomonas aeruginosa* to chlorine-based disinfecting solutions: implications for infection prevention and ward disinfection. J Med Microbiol. 2014; 63(Pt 9):1214-9.
- 27. Pathmanathan SG, Samat NA, Mohamed R. Antimicrobial susceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital. Malays J Med Sci. 2009; 16(2):28-33.
- 28. Judge C, Galvin S, Burke L, Thomas T, Humphreys H, Fitzgerald-Hughes D. Search and you will find: detecting extended-spectrum β-lactamase-producing Klebsiella pneumoniae from a patient's immediate environment. Infect Control Hosp Epidemiol. 2013; 34(5):534-6.

- 29. Girish N, Saileela K, Mohanty SK. Extended Spectrum β-Lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* in neonatal intensive care unit. J Bacteriol Parasitol. 2012; 3:141.
- 30. Weber DJ, Rutala WA. Understanding and preventing transmission of healthcare-associated pathogens due to the contaminated hospital environment. Infect Control Hosp Epidemiol. 2013; 34(5):449-52.