



Revista de Epidemiologia e Controle de Infecção

ISSN: 2238-3360

reciunisc@hotmail.com

Universidade de Santa Cruz do Sul

Brasil

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Prevalence of Nosocomial Infection Microorganisms and the Presence of Antimicrobial Multi-Resistance
Revista de Epidemiologia e Controle de Infecção, vol. 11, núm. 2, 2021, -Junio, pp. 91-96
Universidade de Santa Cruz do Sul
Santa Cruz do Sul, Brasil

DOI: <https://doi.org/10.17058/reci.v11i2.15187>

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Prevalence of Nosocomial Infection Microorganisms and the Presence of Antimicrobial Multi-Resistance

Prevalência de Microrganismos de Infecção Hospitalar e a Presença de Multirresistência Antimicrobiana

Prevalencia de Microorganismos de Infección Hospitalaria y Presencia de Multirresistencia Antimicrobiana

<https://doi.org/10.17058/reci.v11i2.15187>

Received: 13/07/2020

Accepted: 14/01/2021

Available online: 25/06/2021

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ABSTRACT

Background and Objectives: hospitals represent the place where multidrug-resistant bacteria are concentrated and from where they spread within the institution and to the community. Thus, this research aimed to verify the profile of microorganisms related to nosocomial infection and to analyze the existence of multidrug-resistant bacteria at the *Hospital de Caridade de Santo Ângelo/RS*, the largest hospital in the city. **Methods:** hospital infection data from 100 medical records, from August/2016 to March/2017, maintained by the Hospital Infection Control Committee were used. Bacterial identification was carried out by the Microbiology Laboratory of the hospital using morpho-tinting and biochemical methods; and antimicrobial susceptibility was determined by the disk diffusion method. Bacterial multidrug-resistance was considered based on resistance to three or more classes of antimicrobials. **Results:** the three most prevalent microorganisms were *Acinetobacter baumannii* (17%), *Escherichia coli* (16%), and *Staphylococcus aureus* (8%). The hospital unit with the highest number of isolates was the adult Intensive Care Unit, with 41% of the cases. Tracheal secretion, urine, and wound secretion samples provided the highest number of isolates. Sixteen different species/groups of multidrug-resistant bacteria were identified, as follows: *Morganella morganii* (100%), *Hafnia alvei* (100%), *Enterobacter sakazaki* (100%), *Serratia* spp. (100%), *Enterobacter aerogenes* (100%), *Proteus vulgaris* (100%), *Acinetobacter baumannii* (100%), *Klebsiella pneumoniae* (83%), *Enterobacter* spp. (75%), *Klebsiella ozaenae* (66%), coagulase-negative *Staphylococcus* (66%), *Escherichia coli* (56%), *Serratia rubidaea* (50%), *Serratia marcescens* (50%), *Staphylococcus aureus* (37%) e *Pseudomonas aeruginosa* (28%). **Conclusion:** the large number of multidrug-resistant isolates reinforces the importance of isolation and restriction strategies to avoid cross-contamination to inside and outside the hospital.

Descriptors: Cross Infection. Bacteria. Anti-infective agents.

RESUMO

Justificativa e Objetivos: hospitais representam o local onde bactérias resistentes se concentram e a partir de onde se espalham dentro da própria instituição e para a comunidade. Assim, esta pesquisa teve como objetivo traçar o perfil dos microrganismos relacionados à infecção hospitalar e analisar a existência de multirresistência no Hospital de Caridade de Santo Ângelo/RS, hospital de maior porte na cidade. **Métodos:** foram utilizados os dados de infecção hospitalar de 100 prontuários, de agosto/2016 a março/2017, mantidos pela Comissão de Controle de Infecção Hospitalar. A identificação das bactérias foi realizada pelo Laboratório de Microbiologia do próprio hospital através de métodos morfo-tintoriais e bioquímicos, e a sensibilidade foi determinada pelo método de difusão em disco. A multirresistência bacteriana foi considerada a partir da resistência a três ou mais classes de antimicrobianas. **Resultados:** os três microrganismos com maior prevalência foram *Acinetobacter baumannii* (17%), *Escherichia coli* (16%) e *Staphylococcus aureus* (8%). A ala hospitalar com maior número de isolados foi a Unidade de Terapia Intensiva adulta, com 41% dos casos. As amostras que forneceram o maior número de isolados foram: secreção traqueal, urina e secreção de ferida. Foram identificadas 16 diferentes espécies/grupos de bactérias multirresistentes: *Morganella morganii* (100%), *Hafnia alvei* (100%), *Enterobacter sakazaki* (100%), *Serratia* spp. (100%), *Enterobacter aerogenes* (100%), *Proteus vulgaris* (100%), *Acinetobacter baumannii* (100%), *Klebsiella pneumoniae* (83%), *Enterobacter* spp. (75%), *Klebsiella ozaenae* (66%), *Staphylococcus coagulase negativa* (66%), *Escherichia coli* (56%), *Serratia rubidaea* (50%), *Serratia marcescens* (50%), *Staphylococcus aureus* (37%) e *Pseudomonas aeruginosa* (28%). **Conclusão:** o grande número de isolados multirresistentes reforça a importância de estratégias de isolamento e restrição para evitar a contaminação cruzada dentro e até mesmo para fora do hospital.

Descritores: Infecção hospitalar. Bactérias. Anti-infecciosos.

RESUMEN

Justificación y Objetivos: Los hospitales representan el lugar que más concentra bacterias resistentes que se propagan para la comunidad. Por lo tanto, esta investigación tuvo como objetivo esbozar el perfil de microorganismos relacionados con la infección nosocomial y analizar la existencia de resistencia a múltiples fármacos en el Hospital de Caridade de Santo Ângelo (Rio Grande do Sul, Brasil), el más grande de la ciudad. **Métodos:** se utilizaron los datos de infección hospitalaria de 100 registros médicos, referidos al período de agosto/2016 a marzo/2017, mantenidos por la Comisión de Control de Infecciones del Hospital. La identificación de la bacteria fue realizada por el Laboratorio de Microbiología del propio hospital, utilizando métodos morfológicos, colorantes y bioquímicos, y la sensibilidad fue determinada por el método de disco difusión. La multirresistencia bacteriana se consideró basada en la resistencia a tres o más clases de antimicrobianos. **Resultados:** los tres microorganismos más prevalentes fueron: *Acinetobacter baumannii* (17%), *Escherichia coli* (16%) y *Staphylococcus aureus* (8%). El ala del hospital con el mayor número de aislamientos fue la Unidad de Cuidados Intensivos (UCI) para adultos con el 41% de los casos. Las muestras que proporcionaron el mayor número de aislamientos fueron: secreción traqueal, orina y secreción de heridas. Se identificaron 16 especies/grupos diferentes de bacterias multirresistentes: *Morganella morganii* (100%), *Hafnia alvei* (100%), *Enterobacter sakazakii* (100%), *Serratia* spp. (100%), *Enterobacter aerogenes* (100%), *Proteus vulgaris* (100%), *Acinetobacter baumannii* (100%), *Klebsiella pneumoniae* (83%), *Enterobacter* spp. (75%), *Klebsiella ozaenae* (66%), *Staphylococcus coagulase negativa* (66%), *Escherichia coli* (56%), *Serratia rubidaea* (50%), *Serratia marcescens* (50%), *Staphylococcus aureus* (37%) y *Pseudomonas aeruginosa* (28%). **Conclusiones:** la gran cantidad de aislados resistentes a múltiples fármacos refuerza la importancia de las estrategias de aislamiento y restricción para evitar la contaminación cruzada dentro del hospital e incluso fuera del mismo.

Descriptores: Infección Hospitalaria. Bacterias. Antiinfecciosos.

INTRODUCTION

Nosocomial infections (NIs) represent a serious public health concern, as they generate high treatment costs, interdict inpatient units and increase the number of deaths.^{1,2} It is estimated that between 3% and 15% of hospitalized patients in Brazil develop NIs during their hospital stay.³

No hospital is free from the problem, as there is no intervention available that can eradicate NIs; however, a continuous searching for cases reduction should be one of the main establishment goals. With this aim, the Ministry of Health created specific actions to try to prevent and control NI cases. One of them is developing

health policies, which requires the creation of an Infection Control Committee in each hospital. This Committee aims at monitoring NI and controlling the rational use of antimicrobials and germicides.¹

An important aspect that must be considered in NIs is the emergence of multidrug-resistant microbial strains to available antimicrobials, what limit the infections treatment.^{4,5} Monitoring the bacteria responsible for NIs and knowing their susceptibility to antimicrobials allows us to locate the infection sources, verify the level of severity to which patients are subject, and devise strategies to alleviate the problem. In this context, this research aimed to trace the profile of microorganisms related to hospital

infection and analyze the existence of multidrug-resistance in the *Hospital de Caridade de Santo Ângelo/RS*, the largest hospital in the city.

METHODS

Study location and samples

This study was carried out at the *Hospital de Caridade Santo Ângelo*, the largest hospital in the city, with 165 beds, located in northwest Rio Grande do Sul. The Hospital assists 24 cities, covered by the 12th Regional Health Coordination. The study was retrospective and descriptive, carried out from August 2016 to March 2017. Data were collected from the hospital's database provided by the Hospital Infection Control Committee, resulting in 100 medical records analyzed. The population consisted of hospitalized patients who developed NI during hospitalization, regardless of age, gender, race, or social status. NI cases were considered to be those isolated from infections that occurred after a patient was admitted to the hospital and that do not appear in the patient's previous exams.

Microorganisms and susceptibility profile

Microorganism identification and susceptibility profile information were performed by the hospital's Microbiology Laboratory. Conventional methods of isolation and bacterial identification, involving Gram, growth in selective medium, fermentation of sugars, oxidase, and motility were performed.⁶ Of each specimen/sample/site analyzed, only one microorganism was isolated. To determine susceptibility, Kirby-Bauer method (diffusion-on-disk) was used, according to the CLSI standardization (Clinical and Laboratory Standards Institute).⁷ The antimicrobials used were nalidixic acid, amikacin, amoxicillin + clavulanate, ampicillin, ampicillin + sulbactam, azithromycin, aztreonam, cephalothin, cefazolin, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, clarithromycin, clindamycin,

chloramphenicol, erythromycin, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, norfloxacin, oxacillin, penicillin, polymyxin, piperacillin + tazobactam, polymyxin, sulfazotrim, and vancomycin.

Identification of multidrug-resistance bacteria, clinical specimen and hospital unit

For multidrug-resistance analysis of the isolates, the concepts established by Magiorakos *et al.* (2011) were used as reference, in which those isolates resistant to three or more classes of antimicrobials are considered multidrug-resistant.

Moreover, clinical specimen and hospital unit related to NI were identified. Data were tabulated through appropriate coding using Microsoft Excel program.

RESULTS

Analysis of the NI from August/2016 to March/2017 showed the following distribution of cases: August (13%), September (12%), October (12%), November (8%), December (11%), January (13%), February (14%), and March (17%). The 4 most prevalent microorganisms found were *Acinetobacter baumannii* (17%), *Escherichia coli* (16%), *Staphylococcus aureus* (8%), and *Pseudomonas aeruginosa* (7%), which accounted for a total of 48% cases analyzed. Altogether there were 23 species/groups of bacteria found, plus the yeast *Candida albicans* (Figure 1).

In relation to NI cases in each hospital unit, we observed that 41% occurred in the adult ICU; 28% in Unit A (surgical patients); 14% in Unit E (patients in general); 11% in Unit B (patients in general); 3% in the newborn ICU (newborns up to 28 days old); 2% in the Pediatric Unit; and 1% in Unit D (maternity). The prevalence of NI microorganisms in the adult ICU was similar to observed in all hospitals, with *A. baumannii* (21.95%) and *E. coli* (21.95%) being the most frequent. However, *Klebsiella pneumoniae* (9.5%) and *C. albicans* (7.31%) are considered the third and fourth most prevalent, respectively. Meanwhile, *P.*

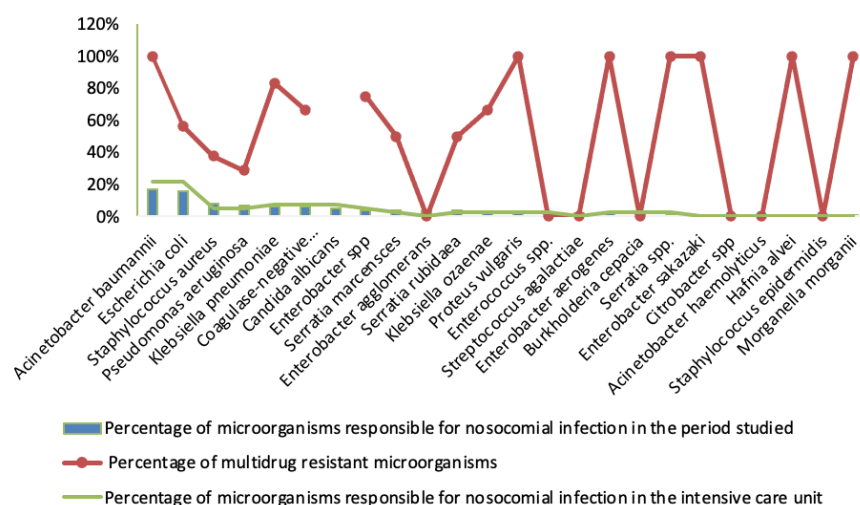


Figure 1. Cases of nosocomial infections verified from August 2016 to March 2017.

Table 1. Microorganisms related to nosocomial infections classified according to hospital unit.

ADULT ICU	NEWBORN ICU	PEDIATRICS	UNIT A	UNIT B	UNIT D	UNIT E
<i>Proteus vulgaris</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>	<i>Acinetobacter baumannii</i>	<i>Acinetobacter baumannii</i>	<i>Enterococcus spp</i>	<i>Klebsiella pneumoniae</i>
<i>Candida albicans</i>	<i>Staphylococcus epidermidis</i>	<i>Serratia marcescens</i>	<i>Pseudomonas aeruginosa</i>	<i>Streptococcus agalactiae</i>		<i>Pseudomonas aeruginosa</i>
<i>Enterobacter spp.</i>			<i>Proteus mirabilis</i>	<i>Escherichia coli</i>		<i>Klebsiella ozaenae</i>
<i>Acinetobacter baumannii</i>			<i>Enterobacter agglomerans</i>	<i>Candida albicans</i>		<i>Enterobacter spp.</i>
<i>Serratia marcescens</i>			<i>Enterobacter spp.</i>	<i>Citrobacter sp</i>		<i>Enterobacter sakazaki</i>
<i>coagulase-negative Staphylococcus</i>			<i>Serratia marcescens</i>	<i>Acinetobacter haemolyticus</i>		<i>Serratia rubidaea</i>
<i>Enterococcus spp.</i>			<i>Candida albicans</i>			<i>Staphylococcus aureus</i>
<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>
<i>Burkholderia cepacia</i>			<i>Escherichia coli</i>			<i>Serratia rubidaea</i>
<i>Klebsiella pneumoniae</i>			<i>Streptococcus agalactiae</i>			<i>Hafnia alvei</i>
			<i>coagulase-negative Staphylococcus</i>			
<i>Pseudomonas aeruginosa</i>			<i>Staphylococcus</i>			
<i>Enterobacter aerogenes</i>			<i>Klebsiella ozaenae</i>			
<i>Serratia rubidaea</i>			<i>Enterobacter aerogenes</i>			
<i>Staphylococcus aureus</i>			<i>Enterobacter agglomerans</i>			
			<i>Morganella morganii</i>			

aeruginosa ranks seven (4.76%) (Figure 1).

Tracheal aspirate (30%), urine (20%), wound secretion (12%), cartilage/sponge/ligament swab (12%), surgical wound (11%), and blood (5%) were the most prevalent clinical sites and specimens providing microorganisms associated with NI. From each site or specimen identified, only one microorganism was isolated.

When we assessed the bacteria in relation to the number of multidrug-resistant antimicrobial classes, we found a large percentage of multidrug-resistant isolates. Sixteen different species/groups of multidrug-resistant bacteria were identified, namely: *Morganella morganii* (100%), *Hafnia alvei* (100%), *Enterobacter sakazaki* (100%), *Serratia spp.* (100%), *Enterobacter aerogenes* (100%), *Proteus vulgaris* (100%), *Acinetobacter baumannii* (100%), *K. pneumoniae* (83%), *Enterobacter spp.* (75%), *K. ozaenae* (66%), coagulase-negative *S.* (66%), *E. coli* (56%), *Serratia rubidaea* (50%), *S. marcescens* (50%), *S. aureus* (37%), and *P. aeruginosa* (28%).

Bacteria were resistance mainly to amoxicillin + clavulanate, ampicillin, cephalothin, ceftazidime, ceftriaxone, sulfazotrim, and cefepime. For these antimicrobials, all isolates of more than two bacterial species were multidrug-resistant.

DISCUSSION

NIs are challenging in developing countries, occurring at high rates. From August to March, we noticed that March (2017) was the month with the highest rate (17%), while November (2016) was the month with the lowest prevalence (8%). It is difficult to say whether these rates are high or low, as NIs vary according to the hospital size, type of care provided, educational institution, public or

private administration, among others. However, experts believe that 1/3 of these infections could be prevented.⁹ The highest rate in March was not significantly higher than in other months, with the exception of November, however, it drew attention. More studies would be conducted, in order to verify what was causing this increase in NI rates in this period.

Regarding the microorganisms involved in the infections, we found 23 different bacteria/groups (Figure 1) and one fungus. *A. baumannii* (17%), *E. coli* (16%), *S. aureus* (8%), and *P. aeruginosa* (7%) appear with a total prevalence of 48%. These bacteria are commonly found also by other researchers as routinely involved in NI.^{2,4,10,11,12}

A. baumannii is considered a prominent nosocomial pathogen due to its high resistance to antimicrobials and disinfectants. Despite the most frequent reservoir of this microorganism being infected patients, widespread environmental contamination is frequently observed, appearing on hospital stretchers, even after cleaning process. *P. aeruginosa* can multiply in soaps and detergents, and these products can often be a source of contamination.^{11,13,14}

E. coli was the second most evidenced bacterium in the study. Currently, this bacterium has received attention due to increasing cases related to multi-drug resistance, both in hospitals and in the community.^{4,15} *S. aureus*, on the other hand, has been considered a nosocomial pathogen that causes high-risk diseases for years. Its infections occur due to colonization of nostrils and skin. This microorganism is characterized by its great capacity to adapt and create mechanisms of resistance to antimicrobials,^{5,16} being responsible for almost half of all deaths caused by antibiotic resistant microorganisms.¹²

It is known that patients hospitalized in an Intensive Care Unit (ICU) are critical and are more vulnerable to NI, compared to other inpatients. There are risk factors

that increase the susceptibility of these patients, namely: clinical severity; existence of surgeries; use of immuno-suppressive and antimicrobial drugs; use of invasive procedures such as central venous catheter and indwelling urinary catheter; use of mechanical ventilators, in addition to intense interaction with the health team; and prolonged hospital stay.^{4,14} NI rates in ICU, in general, range between 18 and 54%.¹⁴ Our study showed a rate of 41%, when compared to other hospital wards, indicating that it requires greater care in an attempt to minimize this rate.

The yeast *Candida albicans* appears as the fourth cause of NI in the ICU. In general, infections caused by *Candida*, represent 80% of all fungal infections in hospitals, appearing contaminating the bloodstream, urinary tract, and surgical wound. This microorganism is found in the microbiota of the human body, however, it becomes pathogenic when there are changes in the host's defense mechanisms or in cases of invasive medical procedures. Among 20 the species of *Candida*, *C. albicans* has the greatest clinical importance due to its gravity and frequency.¹⁷

Lung has been highlighted as an important NI site, especially in ICUs. This fact is due to the necessity of patients to stay in bed, frequent use of sedation and multiple invasive procedures of the airways.^{4,11,18} Of the 100 medical records analyzed, the sample with the highest microbial isolation found was tracheal secretion/aspirate, with 30% of the total number of cases, in agreement with other research in the field.

The second more prevalent site/clinical specimen found in this study, with 20% of the cases, was the urine. Urinary tract infections can account for up to 35% of all hospital-acquired infections, due to the fact that many hospitalized patients are temporarily exposed to indwelling bladder catheterization.^{18,19}

It is also necessary to emphasize that the most severe post-surgical cases are usually directed to ICUs. In these cases, *E. coli* and *Klebsiella* spp are among the bacteria generally involved in these infections.^{2,15} In the present study, 11% of the isolates were from surgical wound exudate and 2% from non-operative, being *E. coli* and *P. aeruginosa* the most prevalent bacteria found. Furthermore, of the 11 cases found in the study, five cases were in the adult ICU; and three, two and one case in Unit E, Unit A and Unit D, respectively.

There is a large proportion of bacteria worldwide resistant to at least one antimicrobial, which is generally used in patient therapy. Patients infected by resistant pathogens have longer hospital stay and require treatment with second and third generation medication, which are more toxic and more expensive, generating higher costs for public health.^{2,4,5,9}

Multidrug-resistance in members of the family *Enterobacteriaceae* has increased vastly lately. Carbapenemase production is more frequent in enterobacteria of the genus *Klebsiella*, *Enterobacter*, *Escherichia*, *Serratia*, *Citrobacter*, *Salmonella*, *Proteus*, and *Morganella*. Carbapenemase attributes resistance to beta-lactam agents such as penicillin, cephalosporins, monobactams, aztreonam, and carbapenems – imipenem. The latter is

considered a broad-spectrum drug used in cases of infections caused by multidrug-resistant bacteria.²⁰ In the present study, two isolates of the genus *Serratia* showed resistance to imipenem, followed by one isolate of *E. coli* and another of *Enterobacter*. All isolates came from different sites, such as tracheal aspirate, femur fragment, blood culture, and surgical wound.

Of the 23 species/groups of bacteria found involved in NI, 16 were multidrug-resistant, with resistance to three or more classes of antimicrobials, which confirms that the hospital can be the focus of a large number of different types of bacterial resistance. This multidrug-resistance encumbers treatment, increasing mortality and morbidity rates, in addition to increasing hospital costs.^{2,21} The selective pressure exerted by the use of various antimicrobials in an attempt to treat the infection, allowing the emergence of new multidrug-resistant bacteria, becoming a dependent cycle. From the hospital, multidrug-resistant microorganisms are taken to the external environment by employees, patients, and visitors. In the community, these microorganisms spread and reach susceptible individuals, increasing mortality, morbidity, and health expenses outside the hospital, confirming that they are a serious public health concern.²¹

Data collected in this study prove the need for measures and strategies to avoid cross-contamination and spread of multidrug-resistant bacteria inside and to outside the hospital.

ACKNOWLEDGMENT

We would like to thank the *Hospital Santo Ângelo's* Infection Control Committee for providing information, which made this work possible.

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AUTHOR' CONTRIBUTIONS

Juliana Rhoden has collected and analyzed the data and partially drafted the text.

Daiane Cristina Prestes has provided the hospital data.

Fábio Pertile has identified the microorganisms and performed the susceptibility to antimicrobials

Joise Wottrich has provided the hospital data.

Letícia Beatriz Matter has carried out the experimental design, partially drafted the article, guided the preparation of graphics, supervised the research, edited the manuscript.