CHAPTER 28

Nosocomial infection in public hospital: Prevalence of multiresistant bacteria, environmental contamination and routine practices

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ABSTRACT
The significant increase in antimicrobial-resistant bacteria is a major public health problem. The Intensive Care Units (ICUs), as they have critically ill patients, are the most susceptible and promoters of hospital infections. Acinetobacter baumannii and Klebsiella pneumoniae are important species that cause hospital infections, associated with resistance mechanisms that make the strains more virulent. The purpose of this article was to perform epidemiological tracking of the most prevalent bacterial strains and seek their involvement with hospital infections in a public hospital in Palmas, Tocantins, Brazil. A retrospective study of nosocomial infections from 2017 to 2019 was carried out. In 2019, screening was carried out on bed surfaces, using a sterile swab. In 2020, suspected cases of hospital infection were registered. The samples were sown in specific media and evaluated the resistance profile. Results: a higher prevalence of A. baumannii was found in Intensive Care Units (70.4%) in the respiratory tract and on surfaces. K. pneumoniae showed a significant increase in the ICU and ICU in 2018 and 2019. Professional practices that could contribute to cross-contamination were observed during collections, with little antisepsis and lack of personal protective equipment being identified. The results reinforce the need to create mechanisms for improvement and continuing education for professionals, as well as effective antisepsis processes, and creating outbreak investigation strategies.

Keywords: Cross infection, Intensive Care Units, Acinetobacter baumannii, Klebsiella pneumoniae.

1 INTRODUCTION

The hospital environment involves the exposure of healthcare professionals and the rest of its staff to a diversity of risks, especially biological hazards, which makes it a place that is prone to diverse types of infections and a significant increase of antimicrobial-resistant bacteria. Therefore, protocols containing control and prevention strategies must be elaborated (Baur et al., 2017). Inside the hospitals, specialized care units as Intensive Care Units (ICUs) and Intermediate Care Units (IMCUs) are more susceptible to cross-contamination considering the presence of patients with critical conditions, invasive procedures such as breathing tubes, central venous catheters, arterial and urinary catheters and the use of broad- spectrum antimicrobials (Liu et al., 2021).

Hospital-acquired infections (HAIs) raised along with the emergence of hospitals themselves and currently they represent an important global public health problem. HAIs are one of the main causes of nosocomial mortality and they can be associated with severe diseases, medical and surgical interventions along complications related to them (Ferraz, 2016; Khan, 2017; Li, 2017). The high frequency of infections and resistant bacteria are serious problems that threaten humanity, as they favor natural selection, there are ecological and epidemiological implications, risk of superinfections, and high number of deaths (Garcia, 2017; Nouri, 2020).

Some factors may favor hospital-acquired infections, such as a patient's weakened immune system, long term hospitalization, inefficient general cleaning, inadequate hand and environmental antisepsis (Costa et al., 2017). To prevent and reduce the risks of contamination, biosecurity measures must be implanted and followed. Measures such as the adoption of rules, secure methods that are appropriated to the maintenance of the health of patients, professionals, and visitors, which help prevent the dissemination of infectious agents, therefore decreasing the risk of developing a nosocomial infection, especially bacterial infections: Stenotrophomonas maltophilia, Klebsiella pneumoniae, Escherichia coli, Streptococcus pneumoniae, Haemophilus influenzae, Acinetobacter baumannii and Staphylococcus aureus, and they may present resistance to several antimicrobials (Zhong, 2020; Andrade, 2021).

Acinetobacter baumannii (A. baumannii) is a non-fermenting gram-negative coccobacilli, considered opportunist beings (Lee et al., 2017). It is an important pathogen that causes infections in many locations, mainly cases of pneumonia associated with mechanical ventilation and/or bacterial sepsis. It also has a high capacity for producing biofilms (Barbapour, 2016; Harding, 2018). Klebsiella pneumoniae (K. pneumoniae) is also an important pathogen related to hospital-acquired infections. It has several resistance mechanisms to drugs. In the last years, the biggest presented problem was due to its resistance to carbapenems. They suffer selective pressure and have transmission by plasmids (Paczosa, 2016; Luo, 2021).

The present work attempted to make an epidemiological screening of the most prevalent clinical bacterial strains and the bacterial resistance profile involved in the hospital-acquired and environmental infections in one of the public hospitals from Tocantins, Brazil, along with the observation of the professional practices.

2 METHODOLOGY

This study is retrospective, prospective, observational and cross-sectional according to Estrela (2018).

2.1 EPIDEMIOLOGY

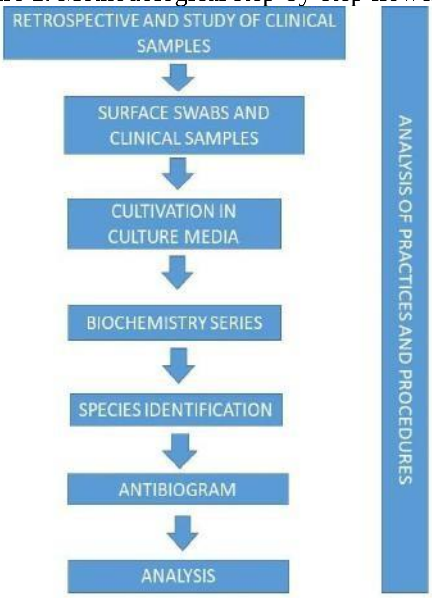
According to the flowchart shown in figure 1, initially, a retrospective study concerning clinical samples was made on the Clinical Analysis Laboratory of the Hospital, in 2017 and 2018, to obtain data about the colonization on the environments of one of the public hospitals in Palmas, both in the Intensive Care Units (ICUs) and Intermediate Care Units (IMCUs). In 2019, information about clinical samples was collected, and concomitantly environmental sampling was made in ICU and IMCU beds, on surfaces that could offer a greater risk of cross contamination, such as the mattress, monitors, countertops, on beds from patients with confirmed bacterial colonization. The sampling was made by using sterile swabs, placed immediately on BHI (Brain-heart infusion) liquid growth medium or Stuart transport medium. The criteria for selecting the beds were made randomly. However, they should present patients with bacterial colonization by resistant strains. On all the surfaces, the samples were collected in duplicates, at the same spot. In the Microbiology laboratory, they were seeded on sheep blood agar, MacConkey agar, and Chromogenic agar, using a Laminar Flow Hood. After the isolation of the colonies, the biochemical test for gram-negative bacteria was made in tubes and the Bactray system by Laborclin. The samples were submitted to the antimicrobial resistance test, by using the Kirby-Bauer disk diffusion test in Mueller Hinton agar. When it was possible, requests were made to obtain clinical samples of the strains that were isolated by the hospital's laboratory, which were stored in -80ºC glycerol, along with the environmental samples.

The antimicrobial profile used on the Hospital's Laboratory for K. pneumoniae was gentamicin, amikacin, ciprofloxacin, ampicillin, amoxicillin+clavulate, piperacillin+tazobactam, aztreonam, cefoxitin, ceftriaxone, ceftazidime, cefepime, meropenem, imipenem, chloramphenicol, sulfamethoxazole/trimethoprim, levofloxacin, and ofloxacin. For A. baumannii, gentamicin, amikacin, ciprofloxacin, meropenem, imipenem, sulfamethoxazole/trimethoprim, and levofloxacin. The environmental samples were also evaluated with regard to their antimicrobial profile and were compared to the strains of the discovered infections.

The sample collection and visits from the team were made on alternate days and schedules, so the observation and the collection could be done at different times and with different teams. In the hospital, during the collections, the practices of the healthcare professionals were observed, in several routine procedures, to check if the preventive measures to hospital-acquired infections, based on the guidelines from both the Health Ministry and the National Agency of Health Surveillance (ANVISA), were being followed. All the inadequate practices and ignored procedures were documented and described in spreadsheets on Excel for further analysis. Collected information that could cause uncertainty was disconsidered.

The project was authorized by the Ethics Committee in Research with human beings UFT, CAAE: 02314818.7.0000.5519.

Figure 1. Methodological step-by-step flowchart.



Source: Authors.

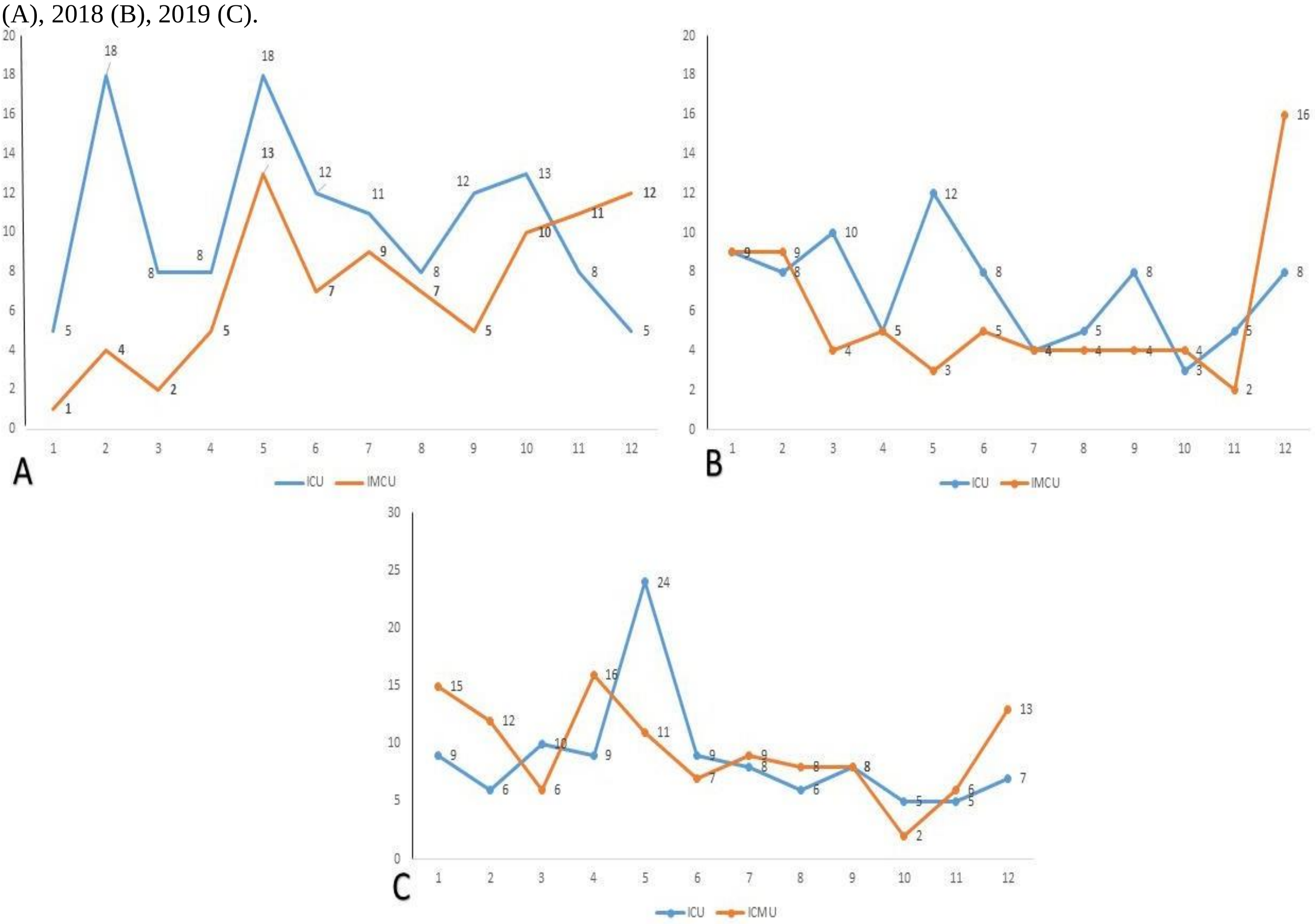
2.2 STATISTICAL PROCESSING

Statistical program Graph Pad Prism 7® was used. For the normality tests, Shapiro-Wilk tests were used and comparisons were made between three independent variables by analysis of variance (ANOVA). For data with normal distribution, the post hoc Tukey test was used to detect the differences between the years, in each intensive care unit. It was considered statistically significant when $p<0.05$.

3 RESULTS

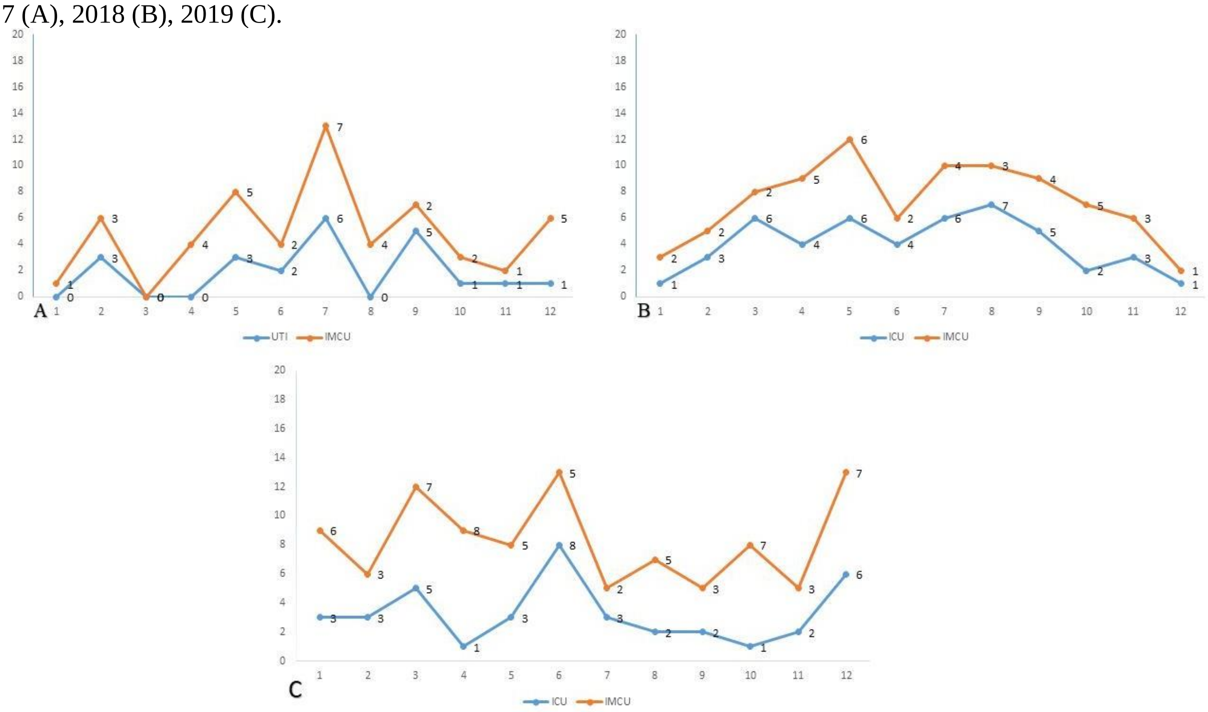
A. baumannii and K. pneumoniae were the species with greater prevalence in the hospital's ICUs and IMCUs. A. baumannii was the most prevalent strain on the clinical samples, presenting a total of 831 cases in the entire hospital in the three years. Especially in the intensive treatment units, it was present in 70,4% of colonized patients cases. ICU with 317 cases (38,2%) and IMCU with 268 cases (32,2%) (Figure 2). K. pneumoniae was isolated in 540 clinical samples in the entire hospital in the three years of research. 109 on ICUs and 136 on IMCUs, totalizing 45,4% (Figure 3).

Figure 2. A. baumannii from the clinic of a Public Hospital in Tocantins, Brazil, in the ICU and IMCU settings, in 2017 (A), 2018 (B), 2019 (C).



Source: Authors.

Figure 3. K. pneumoniae in clinical samples from a Public Hospital in Tocantins Brazil, in the ICU and IMCU settings, in 2017 (A), 2018 (B), 2019 (C).



Source: Authors.

A. baumannii presented, in the three years (2017, 2018, and 2019), an average of 8.8 cases/month, in the ICUs and

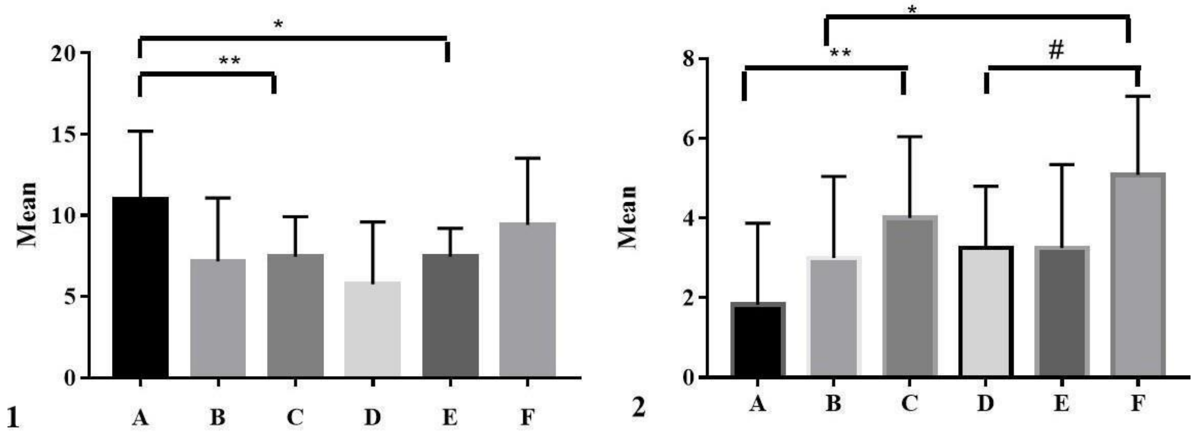
7.44 cases/month in the IMCUs. However, K. pneumoniae obtained a higher average in the IMCUs (3.02 cases/month in the ICUs and 3.78 cases/month in the IMCUs). This number of cases in the ICU can be explained by the greater number of beds available, along with the greater circulation of professionals. It was observed that in the ICU there was a significant decrease from 2017 to 2018 and 2019 in the contamination by A. baumannii. Regarding infections by K. pneumoniae, there was an increase in the ICU in 2018 and the IMCU in 2019, compared to previous years (Table 1; Figure 4).

Table 1. Mean and standard deviation of infections by A. baumannii and K. pneumoniae in the ICU and IMCU, in the years 2017, 2018, and 2019, in a public hospital in Tocantins, Brazil.

Unit Hospital / Bacteria	***A. baumannii***	***K. pneumoniae***
ICU 2017	11,0 +/- 4,19	1,83 +/- 2,03
ICMU 2017	7,16 +/- 3,90	3,0 +/- 2,04
ICU 2018	7,45 +/- 2,46	4,0 +/- 2,04
ICMU 2018	5,75 +/- 3,86	3,25 +/- 1,54
ICU 2019	7,45 +/- 1,75	3,25 +/- 2,09
ICMU 2019	9,41 +/- 4,1	5,0 +/- 1,97

Source: Authors.

Figure 4. Significant differences in contamination between the years 2017 and 2019, in the ICU and the IMCU, of a Public Hospital in Tocantins, Brazil. (1- A. baumannii; 2- K. pneumoniae; A- ICU 2017; B-IMCU 2017; C-ICU 2018; D-IMCU 2018; E-ICU 2019; F-IMCU 2019) * / ** / # significant difference between the groups, p <0.05.



Source: Authors.

As for the distribution of hospital-acquired infections, by topography, it was observed that the site with the highest prevalence of infections by A. baumannii was the respiratory tract, in samples of tracheal aspirate in the average of the three years, in the ICU and the IMCU, together, with average 76.5% in 2017, 64.7% in 2018 and 60.8% in 2019. K. pneumoniae had a higher prevalence in rectal swabs in 2017 (35.5%) and 2018 (33.66%). In 2019, the prevalence was higher in blood culture samples (34.5%), whereas tracheal aspirate appears in third place.

3.1 THE ENVIRONMENTAL SAMPLES

In 37 surface samples of the ICU and IMCU, 21.62% (8) of A. baumannii were isolated, 16.21% (6) in the ICU, and 5.40% (2) in the IMCU. K. pneumoniae was isolated in 5.40% (2) in the ICU only. All strains found had a resistance profile similar to the clinical samples, because, after identification, it was compared to the patient's infection. Making a simple analysis, considering that 5 surfaces are contaminated and have the same probability of transmission, then we would have a 20% chance of cross-transmission, however, there's reason to believe that some places such as mattresses and monitors may offer a greater possibility, as they are being manipulated at all times, followed by manipulation by the patient, which would make this percentage different and another study would need to be carried out. From these positive surface samples, it can be inferred that the failure in antisepsis and/or in the conduct of professionals and visitors can justify the multiple contamination peaks (Table 2).

3.2 OBSERVATIONAL RECORDS

During the visits to make environmental collections, procedures from professionals who work in the ICU and UCI environments were observed. Routine behaviors and negative points in the environment that could favor cross- contamination were observed. The use of adornments and loose hair and little antisepsis of the hands and arms and little or inadequate surface disinfection were especially observed. The combination of inappropriate practices and the presence of microorganisms in the environment favor contamination in general, expanding the possibilities of cross-infection in patients (Table 2).

Table 2. Observational record of routine practices at a public hospital in Tocantins, Brazil.

Record of routine practices	
Daily and terminal disinfection of the room Triple hand hygiene and use of antiseptics	Constant lack of 70% etanol Between the care of one patient and another, the team has no habit of hand antisepsis.
Improper use of surgical aprons and pajamas by the hospital corridors and pantry environment.	Little use of masks.
Poor hygiene of the counters, doors, and handles.	Environmental multi-resistance control is not often performed
No use of ultraviolet light	Bracelets, watches, and rings are not removed by some professionals and academics.
Lack of mats with disinfectants at the entrance to the ICU	Easy access for academics at the UCI and ICU.
Professionals and students who do not tie their hair and do not wear a cap.	

Source: Authors.

4 DISCUSSION

A. baumannii presented bigger variations during the months of 2017, 2018, and 2019, which can represent resistance by those strains in regards to their survival in the environment, and possible flaws on the antisepsis, and control procedures, once those opportunistic pathogens are associated with hospital-acquired infections because of their presence on dry surfaces, and on the hands of professionals from the healthcare unit, which makes cross-contamination more evident and difficult to be controlled (Perez et al., 2020). Bacterial strains have several virulence factors and affect many hospitalized patients in serious conditions. One of the mechanisms that favor the increase in virulence is the increasing use of broad-spectrum antimicrobials, which forces a positive pressure on resistance, increasing the risk of serious infections and without adequate response to available antimicrobials, being directly related to deaths from nosocomial infections. (Yadav et al., 2020). Currently, the increase in the indiscriminate use of broad-spectrum antimicrobials in public hospitals occurs often due to the lack of first-choice antimicrobials in health units, making medical conduct more difficult and forcing the use of more potent drugs at the beginning of treatment. In a recent study, genes related to the transmission of resistance to beta-lactams and the production of carbapenemases were identified in bacteria isolated from clinical samples from a hospital, demonstrating the high dissemination and prevalence of antimicrobial resistance (Ochonska et al., 2021)

K. pneumoniae has been acquiring high resistance, mainly to carbapenems, making treatment strategies increasingly scarce (Zhu et al., 2020). These two species represent a public health problem, as they are contaminating and opportunistic microorganisms and this favors selective pressure since the identification and detection on surfaces close to the patient, coupled with inappropriate practices favor the cross-contamination of multidrug-resistant strains, such as carbapenems (Boral et al., 2019).

Outbreak control strategies may vary according to the characteristics of the hospital and the type of microorganism involved. In a study by Reeme et al. (2019) intervention packages were presented for the control of nosocomial infections in cases of KPC strains, among the measures presented were the precaution of isolation, the presence of a specific room for patients with KPC, environmental antisepsis twice a day, limit on the number of employees, hygiene of the patient's bed, including wheels, and cleaning with bleach, cleaning of surfaces with peroxyacetic acid, cultures, and antibiogram of infections and surfaces, among other measures. The conclusion was that all measures were effective in controlling these infections. All of which are measures recommended by ANVISA, but which are not always used.

Aspects of seasonality could infer contamination since there was a higher prevalence of A. baumannii in April and May, a period of scarcity or no rain, and a higher ambient temperature K. pneumoniae was more prevalent in December, which is when it starts to rain Kim et al. (2018)

demonstrated the relationship between seasonality and the incidence of A. baumannii, which associates temperature fluctuation with the prevalence of this microorganism, finding a positive correlation in this variable, suggesting then, that temperature would facilitate the bacterial growth of gram-negative bacteria, consequently interfering in the virulence increase. This relation with temperature could have another explanation: it could be associated with the cell wall, specifically with glycophospholipid A, which gives the species a greater chance of growth and increased pathogenicity (Raetz et al., 2007).

The topography of infections represents how easily A. baumannii can colonize the respiratory tract and cause pneumonia, since they are microorganisms that can be transmitted by air (aerosols, droplets, secretions), from person to person and that survive a long time on surfaces, on which it can form biofilms, promoting persistent contamination, and favoring selective pressure, resistance to antimicrobials, and the consequent use of plasmids to transmit resistance genes to other strains (Scarcella et al., 2017). Antunes et al. (2014) point out that the most important infections by A. baumannii and with a higher risk of mortality are mechanical ventilation-dependent pneumonia and blood infections. This data corroborates with the findings of this study. The presence of multidrug-resistant strains in the identified isolates fits in the studies that reveal that A. baumannii has acquired resistance to the wide range of antimicrobials in recent years, usually through horizontal transfer of resistance genes.

In a study by Lin et al. (2020) the ability to form biofilms by A. baumannii from clinical isolates was analyzed, which showed a high capacity to form them, with no relation to the susceptibility to antimicrobials. That result promotes our hypothesis of the presence of biofilms by multiresistant A. baumannii on hospital surfaces, leading to persistence and contamination. The biofilm formation ability involved many genes, the efflux pump gene emrA/emrB and two-component system gene baeR were found to be associated with biofilm formation and sub-MIC tigecycline led to increased biofilm formation in A. baumannii for the first time.

Although we found the strain in practically all hospital environments, the environmental analysis focused on the ICU and IMCU, as it is the environment with the greatest demands and care for patients. In 2015, an epidemiological screening study at the same hospital pointed out that K. pneumoniae, P. aeruginosa and A. baumannii were the strains that had the highest frequencies in clinical samples (Baptista et al., 2015). Sohail et al. (2016) detected similarities in Pakistan with this study, as they found 716 clinical cases of A. baumannii in almost three years, presenting multidrug resistance, also observed in our study. Resistance is an important issue to be considered. In Pakistan, they managed to define tigecycline and colistin for treatment, with low resistance.

The last survey of our study observed that in the period from January to June 2020, in the middle of the COVID-19 pandemic, 110 cases of A. baumannii were registered, 25% in the ICU and

49% in the IMCU. When comparing with the same period of 2019, a 32% reduction in the number of identifications by A. baumannii is noticeable, being a small reduction. Regarding K. pneumoniae, 85 cases were registered, 18% in the ICU and 19% in the IMCU, with a reduction of 28% compared to the same period in 2019. This reduction may be associated with possible increases in antisepsis measures and people flow control, reduction in elective surgeries, and increased use of PPE in this period. Despite the reduction, it is important to consider that they are resistant pathogens that favor secondary infections in patients affected by COVID-19. Baptista et al. (2022) in a recent study at the same hospital, it was identified that most secondary infections in patients hospitalized for COVID-19 in the year 2020 occurred in the respiratory tract, and they were caused by multidrug-resistant pathogens, with the most prevalent strains being K. pneumoniae and A. baumannii, which points to the prevalence of the problems identified in our study.

Viral infections predispose patients to other microorganisms such as bacteria. Other epidemics, such as the 1918 outbreak and H1N1 in 2009, led many people to death from cross-contamination, caused mainly by strains of Streptococcus pneumoniae (Cox et al., 2020). Studies conducted by N. Chen (2020) and Zhou (2020), demonstrated that in several deaths by COVID-19 patients had secondary infections of bacterial origin, such as highly resistant A. baumannii and K. pneumoniae.

Notably, strains remain causing infections in several clinical sites, which makes us think that antisepsis measures remain of low quality and that the call for extra care in pandemic times still seems to be deficient. Another possibility is the poor quality of surface antisepsis procedures that are unable to eliminate possible biofilms.

In a study carried out previously, in the same environment, 14 species of bacteria were isolated in articles from surfaces and patients, with emphasis on the clinical sample with the highest contamination, urine culture with a 66% multidrug resistance. Eighteen articles were analyzed, such as benches, hoses, probes, among others. At the end of the analyzes, a contaminating strain was identified, which reinforces the possibility of cross-contamination and the spread of multidrug resistance due to lack of adequate care (Baptista et al., 2015).

In a systematic review by Dresch et al. (2018) S. aureus appears among the strains that contaminate surfaces the most, followed by Staphylococcus Coagulase Negativa (SCN), strains found in the human skin microbiota, P. aeruginosa, K. pneumoniae and A. baumannii. K. Chen et al. (2014) carried out a study on medical records and concluded that 63.5% general units and 83.2% special units had bacterial contamination, making them potential sources of cross-contamination. In this same study, a greater contamination by resistant methicillin-resistant S. aureus (MRSA), responsible for a large part of nosocomial infections, was identified, significantly higher in special units, making it a serious problem in ICUs. Several factors can favor the transmission of microorganisms in the environment, there are records that, in endemic outbreaks, environmental contamination and transfer of bacteria between patients and the environment were found. A lot of scientific evidence states that the hospital can act as an important reservoir of many nosocomial pathogens in different environments, such as surfaces, medical equipment, hands of professionals and visitors and the water system. Furthermore, the frequently inappropriate use of antibiotics causes the selection of multiresistant pathogens, which circulate in the environment (Facciola et al., 2019).

Good practices must be used, as patient safety is a fundamental principle and the reduction of human errors is necessary, for this it is important to adopt International Goals disseminated by the World Health Organization (WHO). The proper use of PPE, taking extra care about invasive procedures, antisepsis, perception are examples of good practices associated with training and retraining that can identify possible occurrences and their causes (Gaiva, 2017; Duarte, 2020).

The hospital environment should provide safety to patients, companions, and staff, however, what has been observed is that many procedures that could prevent cross-contamination and the spread of microorganisms are not performed satisfactorily. The hand hygiene, disinfection procedures and bundles are essential strategies for one of the most important problem of public health. Concurrent cross-infection control strategies are most effective. Alternatives for cleaning and disinfection are fundamental allies for the prevention of hospital infections (Protano et al., 2019).

The apron is an element that allows the spread of microorganisms through the hospital environment, and should only be used in places of biological risks, such as inside the hospital. However, the inappropriate use of it in places such as cafeterias and public environments outside the hospital is commonly observed. Within hospitals, there is little awareness or lack of knowledge of how and where to use it correctly. Studies point to the contamination of clothing, uniforms and coats of health professionals and that favor health-related infections and there are records that microorganisms can survive from 10 to 98 days in clothing, which is made in fabrics such as cotton and polyester (Lima et al., 2020).

4.1 LIMITATIONS OF THE STUDY

Due to preventive measures and the interruption of all research at the Hospital as a result of COVID-19, in 2020 it was not possible to carry out environmental collections. Observational records were not able to be constant in all shifts, due to access restrictions.

5 CONCLUSION

The multi-resistant strains of A. baumannii and K. pneumoniae are difficult to combat, however, it is notable that when preventive actions are added, especially antisepsis, the chance of eliminating microorganisms increases. Intensive care environments deserve special attention due to the structure that favors the spread of microorganisms added to patients in serious situations and thus must have efficient infection control criteria that seek to reduce these processes of cross-contamination. The registration of multidrug-resistant strains indicates selective pressure, making treatment more complicated and, therefore, strategies for controlling the circulation of these microorganisms should be proposed, observing the good practices of employees and users.

Efficient and fast processes that provide adequate disinfection and possible biofilm removals added to the continuous training of the team, making clinical and environmental cultures and antibiograms a routine must be the protocol of the hospital to minimize infections. Creating mandatory workshops for employees' retraining processes, improving information throughout the Hospital are suggestions, however, more studies are essential to help outline strategies. Given the results, the need to develop constant studies to investigate outbreaks, retrospective and cross-sectional, using tracking methods that identify the most prevalent strains and sites is remarkable.

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