

A DOUBLE THREAT: UNVEILING ANTIBIOTIC RESISTANCE IN TRACHEAL ASPIRATE CULTURES OF ICU SARS-COV-2 PATIENTS

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Abstract:

The rapid emergence and spread of the Coronavirus (SARS-CoV-2) in 2019 marked the onset of a global pandemic, posing significant health challenges. SARS-CoV-2 induces a spectrum of symptoms, ranging from coughing and fever to severe cases characterized by dyspnea and hypoxia, often leading to lung failure and fatal outcomes. This study focuses on the pandemic's impact in the state of Santa Catarina, Brazil, where the healthcare system faced a chaotic scenario, characterized by limited vaccination, high demand for ICU beds, and challenges in patient care. By October 2021, Santa Catarina reported 1,200,905 confirmed cases, with a case fatality rate of 1.62%. In the city of Lages, the study's focal point, 28,614 cases were confirmed, presenting unique healthcare challenges. Limited vaccination coverage, overwhelmed healthcare facilities, and the need for patient transfers to other towns or cities further complicated the situation. Notably, the study discusses the opening of new ICU wards in one of Lages' hospitals as a strategic response to the crisis, aiming to provide more specialized care for patients.

Keywords: SARS-CoV-2, Pandemic, Healthcare Challenges, ICU Capacity, Santa Catarina, Brazil

1. Introduction

Infection by the Coronavirus (SARS-CoV-2) began in 2019 in China, where it became a pandemic in a short period of time owing to rapid spread among humans. This disease causes a wide range of symptoms, e.g., coughing, strong headache, fever and, in more severe cases, dyspnea and hypoxia, which can lead to death as a result of lung failure (Moreira, 2020). In Brazil, the first case was notified on February 26, 2020, and the first death occurred on March 17 of the same year in the state of São Paulo. The cases of SARS-CoV-2 increased dramatically; on January 30, 2020, the World Health Organization (WHO) declared it a public health emergency of international concern (World Health Organization, 2020). On March 13, 2021, there was an alarming number of confirmed cases since the beginning of the pandemic in Brazil: 11,363,380 cases out of a total population of 210,147,125 people (Ministério da Saúde, 2021). In the state of Santa Catarina, by October 2021, there were 1,200,905 confirmed cases, of which 1,174,069 have recovered while 19,400 deaths were caused by the coronavirus, i.e., case fatality rate was 1.62%. In the city of Lages (SC), where the present study was conducted, there were 28,614 confirmed cases; while 27,911 have recovered, 529 have died (Secretaria do Estado de Santa Catarina, 2021). It is noteworthy that the pandemic was chaotic in that region when the study began to be developed: public places and non-essential businesses remained closed for days; also, vaccination coverage rate was low, as only frontline COVID health workers and risk groups were allowed to be vaccinated. There was a great demand for ICU beds and hospitalization; patients had to stay at screening facilities - which could offer limited support - while they were waiting for a hospital bed; otherwise, they were transferred to hospitals located in other towns or cities, according to availability. One of the hospitals in Lages opened new ICU wards to offer further support; there was a total of five active wards that offered a more careful and tailored approach to patient treatment.

In view of the above scenario, this study analyzes the reports of tracheal aspirate samples of patients hospitalized in the ICU COVID ward of a hospital in Lages, in the state of Santa Catarina, Brazil, from January to July 2021, with the

objective of: 1) investigating the bacterial profile of positive tracheal aspirate samples submitted to microbiological culture testing, and 2) analyzing the bacterial resistance profile of the most predominant bacterium.

1.1 Manifestation of SARS-CoV-2 infection in patients and possible complications

Most patients affected by SARS-CoV-2 exhibit mild to moderate symptoms; 15% of the cases, approximately, progress to severe pneumonia and 5% of these patients may develop septic shock and/or multiple organ failure. Treatment consists of symptom management and oxygen therapy using mechanical ventilation for patients with respiratory failure (Cao, 2020). On October 25, 2021, there were 243,260,214 confirmed cases of coronavirus worldwide, and confirmed deaths amounted to 4,941,39 deaths (Ministério da Saúde, 2021).

Women account for the highest rate of patients infected by COVID-19, while males showed the highest mortality rate. It is suggested that women have been more resistant to infectious diseases for centuries, as they have regular behavioral habits that help them to maintain good health. Hormonal differences are also a relevant factor because the heterogametic chromosomes of males encourage risk behaviors; thus, men are 17.6% more likely to die early, compared to females. Socioeconomic and sociodemographic conditions also interfere with the mortality rate, as the pandemic in Brazil affected poor males, black people and the elderly to a greater extent (Susuki & Urbano, 2021).

In a portion of the population, the clinical picture of patients infected with COVID-19 evolves very quickly and requires hospitalization; during this period, some important issues may increase the risk of opportunistic pathogen contamination: for example, prehospital administration of broad-spectrum antibiotics, inter-hospital patient transfer, clinical history of previous opportunistic infections and patients undergoing extracorporeal membrane oxygenation (Montrucchio et al., 2020).

Hospital infection is a serious public health problem that, in the presence of unsuccessful treatments or misuse of medication, may lead to relapses, causing complications in patients' clinical picture (Matsuoka et al., 2020). One of the main causes of microbial resistance is the production of specific enzymes against antibiotics, and the former are classified according to the action they exert on the latter (Meyer & Picoli, 2011). When a patient needs hospitalization, data is collected at admission to gather information about the patient's condition. Also, the clinical and the microbiological methods are combined for a more specific and accurate diagnosis of the etiological agent of the respiratory tract of patients using mechanical ventilation (Ranzani et al., 2016).

1.2 Collection and processing of tracheal aspirate samples

Tracheal aspirate samples are collected by nurses or other qualified and experienced health workers. A catheter is inserted into the endotracheal tube with the aid of a swivel adapter and advanced to about 30 cm. After that, it is removed with a sterile disposable secretion collector; the samples are then sent to the laboratory (Corrêa et al., 2014). For processing the samples, two techniques can be applied to detect the etiological agent. The first one is quantitative; execution of the method requires a significant amount and adequate consistency of the sample. If it does not meet the requirements, then the semiquantitative technique can be used, as it is very useful for collecting information about the etiological agent and its antibiotic sensitivity (Sesma et al., 2012).

1.3 Superbug *Klebsiella pneumoniae* and the carbapenemase-producing *Klebsiella pneumoniae* variant

Klebsiella pneumoniae is one of the main Gram-negative opportunistic bacteria, belonging to the family of Enterobacteriaceae. It was first described in 1875 by physician and bacteriologist Edwin Klebs after he analyzed the airways of patients with pneumonia. In 1882, Carl Friedlander described the entire structure of this species (Long et al., 2017), which consists of anaerobic, encapsulated, rod-shaped bacteria associated with excessive mucus secretion, forming mucilaginous and high filamentous colonies, which were shown to be positive in the "string test" (López, Porte & Weitzel, 2020). This bacterium can be found in places such as soil, plants and sewage (Maciel & De Mattos, 2013), and in feces of up to 30% of normal individuals, where they can remain latent in the gastrointestinal tract for a long period of time without causing any damage. However, changes in these conditions, e.g., cases of immunosuppression, can cause a severe disease which can be highly aggressive owing to its ability to recombine chromosomes by sharing plasmid replicons, thus totally altering virulence factors and antimicrobial resistance (Long et al., 2017).

K. pneumoniae may cause nosocomial pneumonia, urinary tract infections (UTI), bacteremia in immunosuppressed patients and even liver abscess, which can progress to hematogenous metastatic dissemination in some isolated situations, and even sepsis (Jun, 2018). The main route of dissemination is through health workers by intra-hospital contact (Silva et al., 2021).

Klebsiella pneumoniae has a variant strain, called carbapenemase-producing *Klebsiella pneumoniae*, popularly known as KPC, a multidrug-resistant bacterium. It was reported for the first time in 1996 in the United States. This bacterium has the ability to hydrolyze the β -lactam ring of carbapenem drugs through the presence of the enzyme b-lactamase. Consequently, it ultimately inactivates rings of the cephalosporins and penicillins classes; and as the gene is located on the plasmid, it easily spreads to other species, promoting the transmission of resistance across bacteria. Strains that show low sensitivity or resistance to imipenem and meropenem are suspected of producing the enzyme carbapenemase (Figueiral & Faria, 2014).

1.4 Bacterial resistance in hospital settings and unfortunate consequences

Resistance happens when microorganisms come into contact with antimicrobials and undergo changes; consequently, drugs become ineffective, which results in prolonged disease, disability and death. Multidrug-resistant pathogens are responsible for increased morbidity and mortality and affect the most fragile patients in the ICU, causing an increase in the cost of medical care, long hospital stays and the need for intensive care (Organização Pan Americana da Saúde, 2021). *K. pneumoniae* shows resistance to 95% of the antibiotics available in the pharmaceutical market. It is considered as one of the main causes of pharmaceutical failure, owing to the production of broad-spectrum β lactamases (Maciel & De Mattos, 2013).

One of the factors that can make the human body more susceptible to the installation of bacteria such as *Klebsiella pneumoniae* and even KPC is the use of corticosteroids. They can increase the risk for a bacterial infection, acting as a “gateway” for opportunistic bacteria. Thus, when a patient’s clinical condition is worsened, diagnosis and monitoring for bacterial infections is of paramount importance, and such infections require adequate treatment in a timely manner (Hosoda et al., 2021). In addition to increasing the probability for installation of opportunistic pathogens, immunosuppressants (corticosteroids) pose risks such as reactivation of previously acquired infections, e.g., hepatitis B, which, in its chronic condition, has been recently associated with a slower elimination of SARS-CoV-2. Thus, there is evidence that recommends traceability for the hepatitis B virus (HBV) through HBsAg and anti-HBc tests (Pérez & Chinesta, 2020).

2. Methods

This study was analyzed by the Human Research Ethics Committee of Centro Universitário Facvest (submission number 50475121.0.0000.5616) and later approved on August 12, 2021 (Decision Report number 4.901.094). A quantitative descriptive study was carried out with data on the total number of semi-quantitative tracheal cultures and their respective antibiograms of patients hospitalized in the ICU COVID-19 ward of a hospital in the city of Lages SC, from January to July, 2021. To be selected for the study, patients should meet the following inclusion criteria: being of legal age, having tested positive for COVID-19, being hospitalized in the ICU and undergoing mechanical ventilation. By contrast, these were the exclusion criteria: underage patients, having tested negative for COVID-19 or still suspected of infection, and hospitalized patients who do not need special care in ICU.

This project was executed only with the laboratory reports. After a request was made to the hospital laboratory, a biomedical scientist on duty accessed the database and provided only the information needed for that study, i.e., the microbiological reports and their respective antibiograms. The researchers were authorized to monitor the processing of this material in the microbiology department, from the arrival of the sample to the release of the report. The protocol begins with the collection of tracheal aspirate samples at the hospital, which must be performed using a sterile secretion collector that is immediately sent to the microbiological department. First, this material is seeded in a triple plate that contains two nutrient media: blood and chocolate, and a selective medium called MacConkey agar; after that, it is incubated in an oven at 36 °C for 24 hours and then an analysis is made of the media where colony growth occurs. If bacterial growth occurred only in the nutrient media, i.e., blood and chocolate, gram staining

is performed to rule out the possibility of this colony being yeast; if it is negative for yeast, the methods of testing gram positive bacteria should be used.

If a bacterium is negative for yeast, the suspected bacterium is reisolated on another blood agar plate, incubated for another 24 hours at 36°C and only on the following day is bacterial identification carried out. The catalase test is performed to differentiate two bacterial families, catalase-positive *Staphylococcus* and catalase-negative *Streptococcus*. However, if bacterial colony growth occurs in the three culture media, the bacterium is gram negative; it must also be re-isolated on another plate for later identification, but in this case only a MacConkey agar plate is used, and it is incubated at 36 °C for 24 hours. The next day, the first test to be performed is the oxidase test; if it is positive, the Bactray III test is performed, and the sample is incubated at 36 °C for 24 hours. If the oxidase test is negative, Bactray I and II tests are performed, and the sample is incubated at 36 °C for 24 hours for further analysis, according to a color scale of each biochemical test.

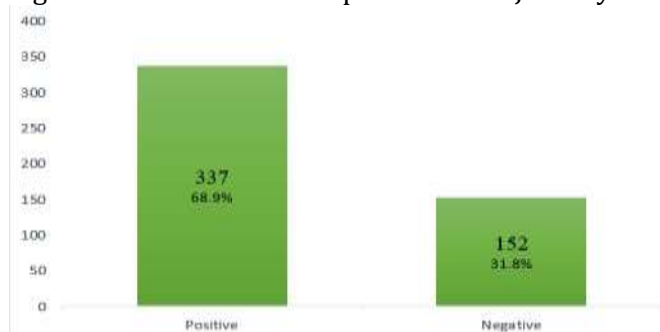
This research was analyzed by the Human Research Ethics Committee of Centro Universitário Facvest (submission number 50475121.0.0000.5616) and later approved on August 12, 2021 (Decision Report number 4.901.094).

A total of 489 laboratory reports were printed. First, they were checked to confirm that every report contained information on bacterial identification and corresponding antibiograms. After that, the reports were sorted into positive and negative samples for bacterial growth. Later, they were separated by sex, age group, months when greater bacterial attack occurred, most predominant bacterium and bacterial resistance of each antibiotic tested. The MS Excel spreadsheet software was used to design graphics for each parameter, which were correlated with information from scientific articles.

3. Results

The results show that 337 (69%) out of the 489 tracheal aspirate samples were positive for microbiological growth while 152 (31%) were negative (Fig. 1).

Fig. 1 Total number of samples between January and July 2021.



Source: Authors.

In the study of Medeiros and Vasco (2020), carried out with an isolated group of 221 patients diagnosed with SARS-CoV-2, 166 did not present a severe condition while 55 did. This smaller portion of individuals (7.7%) had been more affected by bacterial infections, i.e., patients with SARS-CoV-2-mediated infection had a higher rate of coinfections when compared to other patients, who exhibited mild symptoms.

As shown in Figure 2, the highest number of positive samples occurred in March; there was a total of 76 cases. Thus, a correlation can be made with the beginning of vaccination in the city of Lages-SC. According to the data provided by the Epidemiological Surveillance Service, vaccination began on January 19, 2020, but in the first phase, only frontline healthcare workers treating COVID-19 patients and elderly people residing in nursing homes were immunized and, by April, only the population who were 68 years old or older had had access to the vaccine.

Fig. 2 Positive samples classified by month.

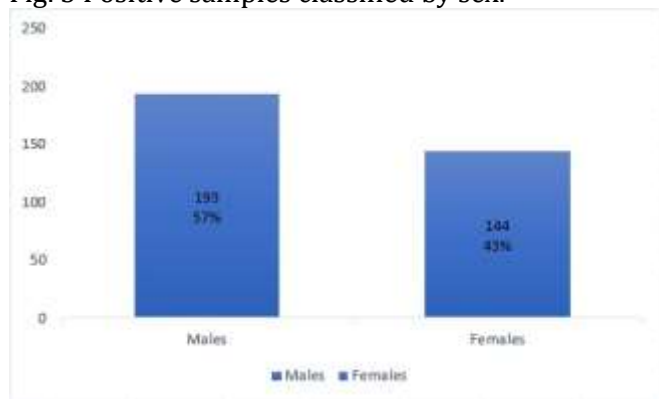


Source: Authors.

Vaccination is the safest option to control the pandemic. Current data show a significant reduction in hospitalizations and deaths in a population that has already received all the doses of the vaccine, especially as of August 2020 (D'Avila & Correa, 2021). This explains the findings; in March, when the number of cases was higher, access to vaccination was still limited and the population had not yet received all the recommended doses for complete immunization, since the only vaccines made available at that time were Coronavac and Astrazeneca.

Of 337 tracheal aspirate samples that had tested positive for microbiological growth, 57% of them belonged to males while 43%, to the females (Fig. 3). There was not a very significant discrepancy between males and females, although the data showed that women accounted for the highest rate of COVID-infected patients, while the mortality rate was higher in males (Susuki, Olak & Urbano, 2021).

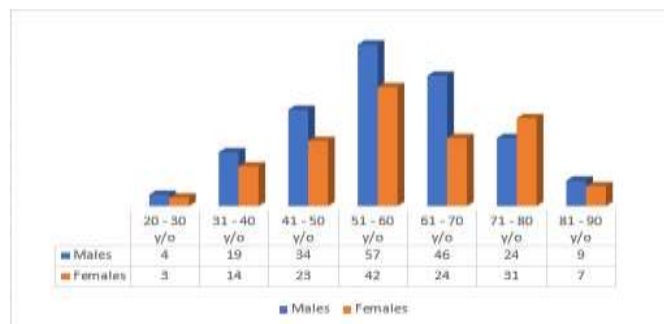
Fig. 3 Positive samples classified by sex.



Source: Authors.

Of all the positive samples for microbiological growth, the highest incidence was in male patients aged 51 to 60 years (Figure 04). As shown on the graphic for sex, there is a slight discrepancy between males and females, i.e., men were the most affected by COVID-19 and, considering data from the literature, men also showed the highest mortality rate, but the most affected age group is the same in both sexes.

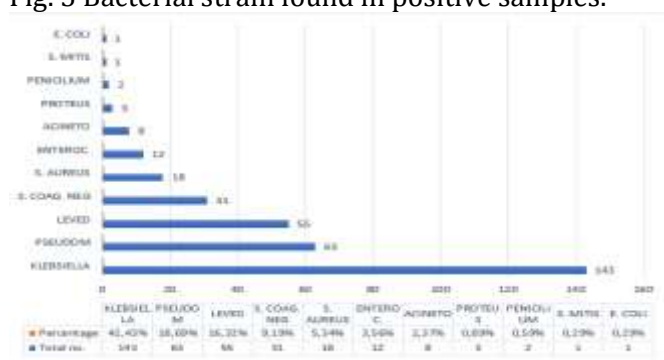
Fig. 4 Positive samples classified by age group.



Source: Authors.

Regarding the bacterial strain of the analyzed samples, the highest incidence was found for the bacterium *Klebsiella pneumoniae* (42.4%), followed by *Pseudomonas aeruginosa*, with a percentage of 18.6%, and yeasts with 16.3% (Figure 5). In Colombia, the main opportunistic pathogens that proved to be multiresistant in isolates from patients hospitalized in health institutions were *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* (Sarmiento et al., 2021).

Fig. 5 Bacterial strain found in positive samples.



Source: Authors.

Klebsiella pneumoniae caused most infections in adult patients hospitalized in ICUs in Brazil in 2017, according to the National Health Surveillance Agency (Anvisa) (Azevedo et al., 2019). Taking into account the number of affected cases compared to others, *Klebsiella pneumoniae* will be addressed in this study. Figure 6 shows a colony of *Klebsiella pneumoniae* in a sample, taken from the hospital, of a patient who was under mechanical ventilation in an ICU COVID ward. This bacterium proved to be the predominant one in the analyzed samples.

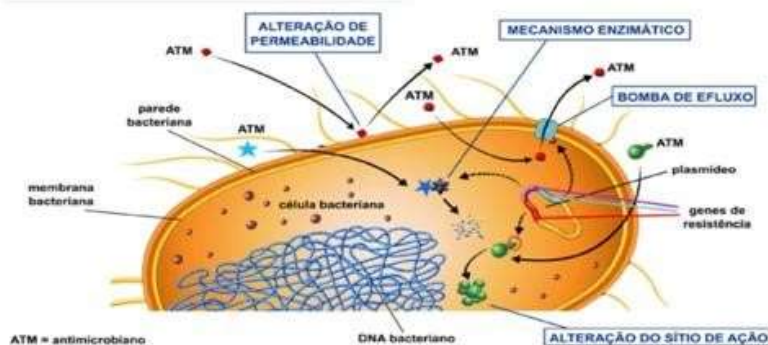
Fig. 6 Colony of *Klebsiella pneumoniae* from the hospital sample.



Source: Authors.

Some bacterial resistance mechanisms used by *Klebsiella pneumoniae* are: absence or decreased expression of some proteins, changes in outer membrane permeability and, consequently, drug absorption; production of chromosomal or broad-spectrum beta-lactamases; efflux pumps; production of carbapenemases (Fig. 7), or even spontaneous mutation and gene recombination which, during conjugation, enable bacteria to share their plasmids and turn from non-mutable into resistant bacteria (Silva et al., 2021). Particular media are used: for example, capsule polysaccharides and lipopolysaccharides, fimbriae, and membrane proteins for iron and nitrogen acquisition for survival during the invasion of human hosts (Li et al., 2014).

Fig. 7 Resistance mechanisms of *Klebsiella pneumoniae*.



Source: Maciel and De

Mattos (2013).

Figure 8 shows an antibiogram of a sample of tracheal aspirate from the hospital ICU COVID ward, performed by the disk diffusion method, which enables the analysis of resistance to most of the antibiotics tested in the colony - a worrying factor when it comes to bacterial resistance.

Fig. 8 Bacterial antibiogram of *Klebsiella pneumoniae* from a sample of bronchial aspirate.



Source: Authors.

Klebsiella pneumoniae has a variant strain called carbapenemase-producing *Klebsiella pneumoniae* (KPC), which is even more aggressive because it produces a carbapenemase enzyme that is capable of degrading drugs used to combat it, such as cephalosporins, penicillins and carbapenems. It is considered as a superbug or multidrugresistant bacterium because it can inactivate most antibiotics available (Silva et al., 2021).

The antimicrobials most commonly used to combat *Klebsiella pneumoniae* belong to the class of penicillins, cephalosporins, aminoglycosides and quinolones, whereas the therapeutic options to combat KPC are limited; polymyxin B, tigecycline and even aminoglycosides can be used (Silva et al., 2021).

Table 1: Antimicrobial resistance of *Klebsiella pneumoniae* as shown in the antibiograms.

Antibiotics	Samples	Percentage
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Ceftazidime	117	83.5%
Ceftriaxone	103	73.5%
Ciprofloxacin	95	67.8%
Ampicillin + Sulbactam	89	63.5%
Piperacillin/Tazobactam	88	62.8%
Cefepime	84	60%
Sulfazotrim	78	55.7%
Levofloxacin	78	55.7%
Amoxicillin + Clavulanic Acid	43	30.7%
Meropenem	29	20.7%
Gentamicin	12	8.5%
Imipenem	7	5%
Amikacin	7	5%
Ertapenem	3	2%

Source: Authors

The 144 positive samples for bacterial growth of *Klebsiella pneumoniae* underwent antibiotic sensitivity testing using the disk diffusion method; approximately 11 different antibiotics were used and incubated in an oven for 24 hours at 36°C for later analysis of halo measurement. Table 1 shows, in descending order and in percentage rates, the antibiotics for which there was most antimicrobial resistance. The data is alarming, since resistance was found to occur for most of the drugs tested to combat the bacteria, especially ceftazidime, accounting for 83.5% of all samples, followed by ceftriaxone, with 73.5%. More than half of the antibiotics being used showed a very high level of resistance; therefore, they are insufficient for bacterial control.

If *Klebsiella pneumoniae* becomes resistant to the antibiotics Imipenem, Meropenem and Ertapenem, it can be considered as a KPC. Table 01 shows the number of resistant cases in six months - a worrying fact taking into account the rate of spread and the severe consequences caused by this strain.

In the laboratory setting, when the antibiogram indicates resistance and/or intermediate resistance to carbapenems (Imipenem, Meropenem and Ertapenem), and if the laboratory in question does not have the installed capacity to screen the presence of carbapenemases, the bacterial strains should be referred to the Central Public Health Laboratory (LACEN/SC) for diagnostic confirmation of the antibiogram result through phenotyping and genotyping. Then, a compulsory notification must be issued, and the Hospital Infection Control Center (CCIH) must be notified. Bacterial isolates to be sent to LACEN must come from pure, recent samples (up to 72 hours after isolation), grown on nutrient agar, in a screw cap tube packed in a rigid-walled biological sample transport box at room temperature (Laboratório Central de Saúde Pública SC, 2016).

4. Discussion

According to the results found in the city of Lages-SC, after six months of research and analysis of almost 500 reports, the study reported alarming numbers for opportunistic infections in a hospital setting, taking into account that only one of the hospitals was analyzed during this period. Of the total number of analyzed samples, 68.9% were positive for the growth of some pathogen, as in the study developed by Lansbury et al. (2020), in which 7% of a total of 3834 patients affected by SARS-CoV-2 had opportunistic infections. Interestingly, the highest rate of positivity was found in patients hospitalized in the ICU; this finding shows how much the immunosuppression caused by the virus is capable of making an environment more fragile and susceptible to the installation of invading microorganisms.

Hospital coinfections are part of a worrying scenario in the clinical condition of patients as they result in a poor prognosis; when patients' health status is more severe, coinfections pose an obstacle to improving the treatment. *Klebsiella pneumoniae* was the bacterium most frequently found in the present study: it was present in 42.43% of all samples. Similarly, in the study carried out by Zhou in 2020 in the city of Wuhan-China, *K. pneumoniae* was the predominant pathogen, present in samples of high-risk ICU patients (Hosoda et al., 2021). The cohort density for the bacterium *K. pneumoniae* increased significantly during the pandemic when compared to the pre-pandemic period (Gaspar, Bollela & Martinez, 2021).

K pneumoniae is a highly aggressive bacterium, with high power of dissemination and mutation, and it has a variant strain called carbapenemase-producing *Klebsiella pneumoniae*, popularly known as KPC, which is extremely resistant to previously developed drugs. As soon as there is a suspected case, all strains found in different body fluids have their genome sequenced; in cases of confirmed KPC, the samples showed genes encoding beta lactamases, and such genes increase resistance to aminoglycosides, quinolones, macrolides, sulfonamine, tetracycline, trimethoprim and fosfomycin. In the study of Montrucchio et al. (2020), carried out with ICU COVID patients, 7 out of 35

participants tested positive for KPC. Their sample was considered as young and free from most risk factors, except obesity, a fact that shows how much this strain can be invasive and opportunistic.

The number of samples from this study that showed resistance to the antibiotics imipenem, meropenem and ertapenem, which are considered as possible KPC, can be explained by the beginning of the pandemic: there was a high demand for inpatient beds, and the priority at that time was to isolate patients affected by COVID-19. Therefore, the impossibility of containing patients with opportunistic infections may have caused cross-transmission, leading to a potential spread (Arcari et al., 2021).

A factor that can also be considered as an aggravating factor for the installation of infections is the use of corticosteroids in patients with SARS-CoV-2. The positive results of administering this drug were evidenced in the RECOVERY study: using dexamethasone 6 mg a day considerably reduced the rate of mortality in patients under mechanical ventilation and increased the probability of survival by about 28 days (González-Castro et al., 2021). However, corticosteroids are immunosuppressive drugs that can cause damage; for example, they may lead to superinfections, hyperglycemia and even the reactivation of some pathologies.

5. Conclusions

Taking into account the six-month study period, the data reported in this study is alarming and reveals a worrying scenario in terms of bacterial resistance in hospital settings, since it is a condition that is becoming progressively worse and can cause the death of a large number of patients.

Klebsiella pneumoniae was the most predominant bacterium in the study samples; in addition to suspected cases of the carbapenemase-producing strain *Klebsiella pneumoniae*, the presence of this enzyme points to a critical scenario, both in terms of antibiotic therapy, whose options are extremely limited, and rapid dissemination of enzymes that lead to resistance through plasmid sharing.

The CCIH monitors opportunistic infections in hospitals; however, data collection has not been performed with such a large amount of information over a larger time span. For this reason, these findings are relevant because they provide further insights into the problem and enable appropriate measures to minimize damage.

Abbreviations

CCIH – Hospital Infection Control Center

COVID-19 – Coronavirus disease 2019

HBc – Hepatitis B core antibody

HBSAg – Hepatitis B virus surface antigen

HBV – Hepatitis B virus

ICU – Intensive care unit

KPC – *Klebsiella pneumoniae* carbapenemase

LACEN – Central Public Health Laboratory

SARS-CoV-2 – Severe acute respiratory syndrome coronavirus 2

WHO – World Health Organization

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