Presence of carbapenemases in clinical isolates of *Klebsiella pneumoniae* in Caleta Olivia, Santa Cruz, Argentina

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Abstract
In this work, the presence of carbapenemase-producing *Klebsiella pneumoniae* was studied in 208 patients admitted to a clinic in the city of Caleta Olivia, Santa Cruz, Argentina, during the months of March to August 2019. Rectal swab samples that were seeded in CHROMagar KPC medium were used. From the sowing, 26 unique isolates of metallic blue colonies were obtained, confirming the presence of *K. pneumoniae* in 100% of the cases. These 26 isolates were positive for the production of KPC-type carbapenemases (12.5% of the total). 77% of the 26 colonized patients progressed to the infection and died during hospitalization. No relationship was found between the presence of KPC and the origin, pathology of admission to hospitalization, age or sex of the patients analyzed. A relationship was found between the presence of KPC and risk factors such as prolonged hospitalization and admission to the Intensive Care Unit, as well as high white blood cell count and low hematocrit. On a marginal basis, two other associated factors were antibiotic treatment and male gender. This work confirms, the presence of KPC-type carbapenemase producing *K. pneumoniae* in rectal swab samples in this Patagonian region of Argentina.
Keywords multiresistant bacteria; nosocomial infection; antibiotic resistance; beta-lactamases

The carbapenemase-producing Enterobacteriaceae (EPC) have become very important in the world. Their rapid dissemination, their difficult control and the scarcity of therapeutic options for the management of infections are factors that have allowed these microorganisms to be the cause of numerous deaths at the hospital level (Rocha Afonso & cols. 2022; Cejas & cols., 2022; Muñoz & cols., 2019).

The genes encoding them are found on genetic elements such as plasmids and transposons, resulting in their rapid spread and frequent transfer of multiple antibiotic resistance. However, the implementation of epidemiological surveillance programs can impact the reduction of incidence (Rocha Afonso & cols. 2022; Lossa & cols., 2008). To a large extent, the success of surveillance programs is based on the early detection of patients colonized by multiresistant bacteria, since they constitute a considerable risk factor for the subsequent acquisition of infections associated with health care and constitute a of the main routes of propagation of said microorganisms within the institution.

The body sites with the highest rate of colonization are the respiratory tract, due to the use of invasive devices (mechanical ventilation) and the gastrointestinal tract, which is considered the most relevant. The gastrointestinal tract is also the main reservoir of multiresistant microorganisms (Santolin & cols., 2017).

To limit the presence of multiresistant pathogens in the hospital setting, the United States Centers for Disease Control and Prevention (CDC) has proposed early detection systems for patients with gastrointestinal colonization through active surveillance and the implementation of infection control measures (CDC, 2009). Likewise, the Argentine Health Care Infection Control Practices Advisory Committee (HICPAC) recommends a series of strategies for the control of CLD in patients hospitalized in acute health centers. These include performing surveillance cultures on all patients admitted to hospital from areas with a high prevalence of carbapenemases (CDC, 2009)

Currently, KPC-type carbapenemase-producing Klebsiella pneumoniae (carbapenemase-producing K. pneumoniae) is the most frequently found CLD species (López et al., 2011; Carmeli et al., 2010). KPCs are of molecular class A and the most prevalent worldwide. These enzymes hydrolyze carbapenems and confer a high level of resistance to all beta-lactams (Tuon et al., 2012).

Klebsiella pneumoniae is resistant to most available antibiotics and the infections it causes have been associated with high morbidity and mortality rates, particularly in patients with prolonged hospitalization, who have received multiple broad-spectrum antibiotic regimens, critically ill patients, and exposed to invasive devices, for example ventilators or central venous catheters (Kohler & cols., 2017).

Although it is isolated as the causal agent of urinary tract infections (UTI) and pneumonia, it is fundamentally an opportunistic pathogen and as such one of the main causes of nosocomial infections. (Echeverri-Toro & Cataño-Correa, 2010). It is processed by the droplets of pflüge and the hands of the staff.

K. pneumoniae has been reported in pneumonia, bacteremia, meningitis, UTI, severe enteritis, soft tissue infections, acute anterior uveitis, biliary tract infections, liver abscesses, peritonitis, and wound infections (Lopardo, Predari & Vay, 2016, Munoz & cols., 2019). This microorganism shows a high capacity for dissemination, which has caused countless hospital outbreaks and the resulting increase in morbidity and mortality.

Another characteristic of this species is its ability to acquire resistance genes, which is why they are currently among the 10 main pathogenic bacteria that cause hospital infections (Lopardo, Predari & Vay, 2016).

It is a pandemic strain, although it was initially described in 1996 in North Carolina, United States, and since then it has been routinely recovered in hospitals in New York and New Jersey, spreading to several countries around the world (Lomaestro & cols ., 2006). It has also been described in Brazil, China, Colombia, Norway, the United Kingdom, India, Sweden (CDC, 2009), Italy, and Finland (Pasteran & cols., 2016). In South America, it was reported for the first time in Colombia in 2006 (López & cols, 2011). In Argentina, the first detection of KPC in Enterobacteriaceae was in 2006, where it was detected in K. pneumoniae and in Citrobacter freundii from the same patient (Pasteran & cols., 2016). Cases have been reported in different provinces (WHONET, 2019).

KPC-type carbapenemase is found mainly in K. pneumoniae (86% of cases) and its presence has also been reported in E. coli, C. freundii, Serratia marcescens, and Enterobacter cloacae (De Belder, 2019; WHONET, 2019) .

Those hospitalized patients who are colonized by positive KPC enterobacteria and who were not detected have been considered "reservoirs of transmission during nosocomial outbreaks" (De Sanctis & cols., 2018).

The state of colonization represents one of the main risk factors for the development of infections (Echeverri, Atehortúa & Robledo, 2009). Various studies report an incidence of KPC enterobacteria infections of 10% in colonized patients (Hoyos Mallecot, 2015)

The most important intervention measures that have been shown to control outbreaks of this type of infection are: optimal adherence to handwashing, patient isolation measures, control of the use of antimicrobials, particularly in the ICU, and lastly, vigilance. with swabs and rectal cultures to identify colonized patients in a timely manner and be able to take control measures efficiently (Kohler & cols, 2017).

Although, the WHONET-ARGENTINA 2019 Network has described strains of K. pneumoniae resistant to carbapenems in blood and urine samples in the province of Santa Cruz; In our environment, the prevalence of K. pneumoniae KPC in rectal swab samples is not known, nor is there a possible association between risk factors and rectal colonization by KPC in patients hospitalized at a Clinic in Caleta Olivia, Santa Cruz (WHONET, 2919).

The objective of this work was to analyze the colonization by Klebsiella pneumoniae, producer of KPC-type carbapenemases, in patients hospitalized in a clinic in the City of Caleta Olivia, Santa Cruz, Argentina, and to identify possible risk factors associated with this colonization.

Materials and methods

A retrospective, descriptive observational study of rectal colonization by K. pneumoniae, producer of KPC-type carbapenemases, was carried out on a population of 208 patients admitted to Clínica Cruz del Sur from March to August 2019. The data sources used were the medical records of the patients. patients and the results of the institution's Clinical Analysis Laboratory.

Inclusion criteria

Patients older than 18 years, with previous hospitalization for more than 48 h in the last 6 months and as exclusion criteria patients who presented previous isolation due to multiresistant microorganisms in the previous 6 months or patients with hospitalization, surgery, and/or or antimicrobial treatment with carbapenems in the last 3 months.

Samples and Processing

Rectal swabs from each patient were taken in duplicate, using Stuart transport medium Dacron swabs to be taken to the laboratory for processing. They were taken upon admission to hospitalization and, if negative, weekly in the Intensive Care Unit or every 15 days in the general ward. Rectal swabs were sown in CHROMagar KPC medium (Benton-Dickinson, United States) incubated for 24 h at 35 °C in an aerobic atmosphere, and positive cultures that could indicate the presence of carbapenemases were studied. This medium contains a chromogenic compound with the addition of a supplement that inhibits the growth of gram positive and negative bacteria sensitive to carbapenems. Colonies are visualized with different colors according to their specific enzymatic properties. In this medium, colonies of K. pneumoniae stain blue.

Colonies that grew metallic blue (Klebsiella spp, Enterobacter spp, Citrobacter spp.) were typed by conventional methods (Lopardo, Predari & Vay, 2016). These included Triple Sugar Iron Agar (TSI Agar), Simmons Citrate Agar, Lysine Iron Agar, Urea Agar, and SIM Medium (Hydrogen Sulfide, Indole, Mobility; Britania, Argentina).

Detection of carbapenemases

The tests for the search for KPC (and other acquired carbapenemases) was the one proposed by the INEI-ANLIS “Dr. Carlos G. Malbrán”, Buenos Aires, agreed upon by the Latin American Antimicrobial Resistance Surveillance Network (RELAVRA), which is fundamentally based on the inhibitory effect of 3 amino-phenyl boronic acid on group A serine-carbapenemases, and antimicrobial agents. Zn chelators on metalloenzymes, using the size of the zone of inhibition of imipenem by the disc diffusion method. According to what is suggested by the "Bacteriology Quality Control Program" (WHONET, 2019), it is proposed to consider as suspected of having carbapenemases all strains with carbapenem inhibition halos $\leq$ 22 mm, within the category of "no sensitive (intermediate or resistant). 10 µg discs with Imipenem, Meropenem and Ertapenem (Benton-Dickinson, United States) were used

Mueller-Hinton agar plates (Britania, Argentina) were swabbed with a 0.5 McFarland suspension of the isolate under study. Antibiotic discs were placed and incubated in an oven at 35 °C for 24 h, after which the inhibition halos were measured.

The synergy test between carbapenems and 3-aminiphenylboronic acid (ABP, 300 µg) was carried out by means of the double disk diffusion method, and between carbapenems and ethylenediamine tetraacetic acid (EDTA, 372 µg)/sodium mercapto acetate. (SMA, 900 µg), for the search for metallobeta-lactamases (MBLs). When the inhibition halos of Imipenem and Meropenem were available from the primary antibiogram, adjusted distances between the 13-mm discs were used (WHONET, 2019). Enlargement of the carbapenem inhibition halo towards the side of the inhibitor disc was interpreted as a positive result.

Additionally, the Britania Resist-3 O.O.K.® Kit was used, based on the sensitization of a nitrocellulose membrane through the use of monoclonal antibodies directed against an epitope of the carbapenemases OXA 48, OXA 163 and KPC (Pasteran & cols., 2016).

Risk analysis

The patients were divided into two groups, depending on whether or not their isolates had carbapenemases. The medical records for each patient were reviewed and the following variables were analyzed: demographic data (age, sex, place of residence), underlying pathology and/or diagnosis, white blood cell levels, hematocrit, and risk factors (prolonged hospitalization, hospitalization in the Intensive Care Unit, invasive devices, immunosuppression and antibiotic treatment). Long-term hospitalization was considered to be longer than 25 days, according to the definition of the United States health services (https://www.cms.gov). For the purposes of this work, the reference values of our laboratory for white blood cells and hematocrit were used, grouping them into High (above 10,000/mm3), Medium or Normal (between 4,000-10,000/mm3) and Low (below 4,000/mm3). For Hematocrit, the values were: High >54 or >48%; Medium 54-40 or 48-37% and Low <40 or <37% for men and women respectively. Below 21%, a critical value was considered to initiate transfusion.

Statistical analysis: The variables: age, sex, place of residence, risk factors, pathology and/or hospitalization diagnosis, leukocyte and hematocrit values and, in addition, presence and/or absence of K. pneumoniae resistance to carbapenems were subjected to principal component analysis using R software (https://www.r-project.org/). The data that refer to normal values were excluded, since they do not add information (for example, if the white blood cells are neither high nor low, both columns will be zero and that combination will correspond to the normal value). For the comparison of frequencies between patients with KPC or without KPC, a Chi2 test with Yates correction was performed, using OPENEPI (www.openepi.org).

Ethical aspects

The study did not represent any risk and did not involve tests on humans or animals. All study data were treated with maximum confidentiality, anonymously, and with access restricted only to the authors, in accordance with current legal regulations, Argentine National Law on the protection of personal data 25,326. Personal data was coded by a numerical system. As this was a retrospective observational study, it was not considered necessary to obtain signed consent from the patients analyzed.

Results

Demographic characteristics and pathologies of patients

The 208 patients who met the inclusion criteria entered the Cruz del Sur Clinic from various locations in the province of Santa Cruz, while five were from different locations in the adjoining province of Chubut. 65.4% came from Caleta Olivia, the city where the Clinic is located. 47.7% (n=93) of the patients were female.

42 pathologies and/or admission diagnoses were recorded, the most frequent being: Oncological (31), Community Acquired Pneumonia (CAP) (23) and Heart Failure (HF) (19) representing 30.2% of the total number of cases. the same (Table 1)

Table 1. Pathologies of the 208 patients.

Pathology	n	Pathology	n	Pathology	n
Oncologic	31	Cellulitis	4	Traumatism	2
Community-acquired pneumonia	23	EPOC	4	Abscess	1
Cardiac insuficience	19	Fracture	4	Angina Pecho	1
Cerebrovascular accident	11	Lumbar/cervical colection	3	Bilirrhagia	1
Renal failure	11	Anemia	3	Lung edema	1
Abdominal Pain	10	Deshidratation	3	Hemiplegia	1
Acute abdomen	8	Gastrointestinal bleeding	3	Hypertension	1
Diabetes	7	Angor	2	Hiponatremic	1
Respiratory Insuficience	7	Cirrhosis	2	Deep venous thrombosis	1
Venous thrombosis	6	Colelitiasis	2	Neutropenia	1
Pancreatitis	6	Precordial pain	2	Choledocian syndrome	1
Chirurgic infection	6	Febrile syndrome	2	Mastitis	1
Acute miocardic infart	5	Meningitis	2	Tiroidectomy	1
Sepsis	5	Hernia	2	Vomiting	1

Biochemical Analysis

56.7% of the patients presented High values of white blood cells and few, less than 6%, Low values (Table 2). The Hematocrit values of the analyzed patients were distributed between Normal and Low (Table 2)

Risk factor's

Risk factors were observed in all the patients analyzed in this study. 70.7% of them received prolonged hospitalization and 61.1% were admitted to the Intensive

Care Unit. In 100% of the patients, the presence of invasive devices was observed, such as: central venous access, bladder catheter, nasogastric tube, MRA, etc. 78.4%, which represents 163 patients, were immunosuppressed and 31 of them were receiving cancer treatment. 77.4% of patients received antibiotic treatment during hospitalization.

Table 2. White blood cell and hematocrit values of 208 patients analyzed.

	Value	n	%
White cell blood	High	118	56.7
	Normal	78	37.5
	Low	12	5.8
Hematocrit	High	0	0.0
	Normal	117	56.3
	Low	91	43.8

Microbiological isolation and determination of carbapenemase production

From the sowing of the rectal swabs in CHROMagar KPC medium (Figure 1A), single isolates of metallic blue colonies were obtained, as in samples 666 and 667. Other samples, on the contrary, were negative (such as 668, 669, 670 and 671).

Conventional biochemical typing tests were performed on these isolates, confirming the presence of K. pneumoniae in 100% (n=26) of the positive cultures.

These cultures were studied by phenotypic assays for the detection of carbapenemases. Figure 1B shows the positive synergy test with 3-amino phenyl boronic acid and negative with EDTA, confirming the presence of class A carbapenemases. Next, the Kit Resist-3 O.O.K.® immunochromatographic technique showed the production of KPC-type carbapenemases in the 26 positive isolates (100%, Figure 1C and 1D), none for OXA-48 and none for OXA-163. This represents 12.5% of the total samples analyzed

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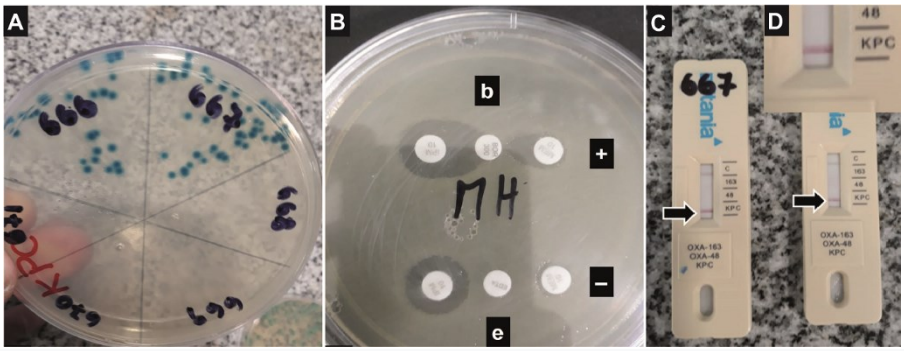

Figure 1. *Positive cultures in CHROMagar KPC medium and carbapenemase tests. (A) Presence of metallic blue colonies (K. pneumoniae, in samples 666 and 667) and negative cultures (samples 668, 669, 670 and 671). (B) Synergy test with amino phenyl boronic acid and EDTA on Mueller-Hinton agar. Note the synergistic effect (+) with amino phenyl boronic acid (b), but absent (-) with EDTA (e). (C) Kit Resist-3 O.O.K from Britania with positive results for the production of KPC-type carbapenemases (arrows). (D) Inset with greater detail of the positive signal*.

Characteristics of patients colonized by KPC

Regarding gender, 57.7% (n= 15) of the patients were men and 42.3% (n= 11) women, with a mean age of 61.8 ± 13.6 years. In 73% of the cases, the age group was over 60 years old. 92.30% (n= 24) of the patients came from home and 100% (n= 26) were found to be colonized in the Intensive Care Unit. No relationship was found between the origin of the patients and colonization by KPC.

When analyzing the carrier state and comparing it with the pathologies of each patient, no association was found between the pathologies and rectal colonization by K. pneumoniae, producer of KPC-type carbapenemases.

The relationship between the risk factors and the levels of white blood cells and hematocrit between the group of patients with and without KPC was analyzed. Prolonged hospitalization and admission to the Intensive Care Unit were the risk factors where a significant difference was observed (0.002 and 0.023, respectively), while the remaining factors were not significant.

|Regarding the values of white blood cells and hematocrit, there were significant differences between the group of patients with KPC and without KPC for the High and Medium levels of white blood cells (0.001 and 0.002 respectively) and the Medium and Low levels of hematocrit (0.001 and 0.001 respectively). Most KPC patients had very low hematocrit levels, in the range of 21-27%.

Principal component analysis was performed to assess whether there was a correlation between the presence or absence of KPC and the variables studied

(Figure 2). There was remaining variability at least up to component 6, of the 10 components analyzed. The first component has major contributions from High White Blood Cells, Low Hematocrit and Risk Factor "A" (Prolonged Hospitalization). The second component has a majority contribution of Risk Factor "B" (Internment in Intensive Care Unit) and to a lesser extent the male gender and Risk Factor "A" (Prolonged Hospitalization). These two components together explain 44.09% of the variance.

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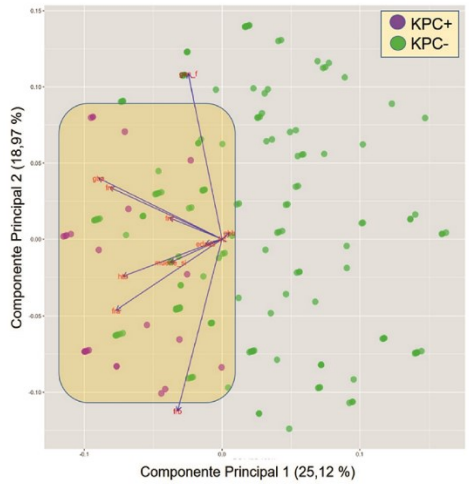

Figure 2. *Principal component analysis of the presence (+) or absence (-) of KPC in relation to the other parameters studied*

Of the 26 patients colonized by K. pneumoniae KPC, 20 developed clinically evident infection, that is; 77%, which represents 9.6% of the total number of patients analyzed.

The sites of infection were: blood (7), respiratory tract (6), abdomen (4), and urinary tract (3). These 20 patients died during hospitalization.

Discussion

Infections measured by CPE increase considerably and are associated with high mortality and morbidity rates, especially in patients with prolonged hospital stays or in critically ill patients (Rocha Afonso & cols., 2022; Schechner & cols., 2012; Panagea & cols., 2010; Giani & cols., 2012).

Because they spread quickly and easily, it is necessary to implement a program of preventive measures and isolation that is appropriate to the type of clinical establishment. This program must involve active surveillance, according to international guidelines (Schechner & cols., 2012). The implementation of epidemiological surveillance programs can reduce the infection rate up to 32% (Pujol & Limón, 2013).

In the present study, out of a total of 208 patients analyzed, 12.5% (n=26) of patients colonized by KPC were identified. The risk of colonization considered in the literature is 5% and the risk of infection in colonized patients is between 10% and 30%, with a high associated mortality (Paño Pardo & cols., 2014).

The worldwide prevalence varies in different geographical regions, most of them acquired nosocomially, and with rates between 7.5 and 44%. The KPC rate has been 44% in Latin America, 22.4% in Asia/West Pacific, 13.3% in Europe, and 7.5% in the United States 6.7% (Melgarejo Touchet & cols ., 2021; Cejas & cols., 2022; Valdés Espino & cols, 2018; Muñoz & cols., 2019).

In a descriptive retrospective study carried out in Argentina, Rivero & cols. (2017) analyzed the prevalence of KPC colonization in patients in critical care units, finding an average prevalence of 1.5% over 3 years. These results differ from this study, where 12.5% of patients colonized by KPC were identified.

On the other hand, Echavarría & cols (2017) carried out a surveillance study at a University Hospital in Buenos Aires in order to determine the prevalence of colonization by carbapenemase-producing K. pneumoniae strains. Two prevalence cuts were made where rates of up to 25% were found, a situation twice as high as in our study.

Additionally, a descriptive observational study, carried out at the Argerich Hospital located in Buenos Aires; Córdova & cols (2012) refer to 10% of patients colonized by K. pneumoniae KPC. These results are similar to those reported in this work.

The situation in Paraguay is similar to that reported in Argentina, as indicated by two studies from 2017 and 2021 (Ortiz & cols., 2022; Melgarejo Touchet & cols., 2021).

For the detection of these KPC-producing strains, different methods have been developed based on chromogenic media, molecular tests and techniques such as laser nephelometry that detect KPC (Alizadeh, Rezaee & Kafil, 2018). Additionally, immunochromatographic tests allow us to differentiate the type of carbapenemase present, with a reported sensitivity and specificity of 100% in various studies compared with molecular biology techniques (Wareham & Abdul, 2019).

In this investigation, 26 of 208 patients studied (12.5%) presented colonization by K. pneumoniae type KPC. 100% of the K. pneumoniae isolates studied showed positive results for the synergy test with boronic acid and negative with EDTA. By the Resist-3 O.O.K.® immunochromatographic technique, the production of KPC-type carbapenemases was confirmed in 100% of the isolates.

Molecular techniques are the gold standard for confirming the presence of carbapenemase genes. However, these techniques are expensive, require equipment, supplies and specialized personnel and therefore their use is restricted to relatively few institutions. For this reason, different phenotypic assays have been proposed for the presumptive detection of carbapenemases in clinical isolates.

The method of synergy between carbapenem discs and APB was highly specific since no false positive results were obtained. However, to achieve maximum sensitivity, the distance had to be adjusted according to the inhibition diameter of the carbapenems. By adjusting the distance between disks to 13 mm (center to center), a synergistic effect between the ABP disk and the carbapenems could be seen in all positive KPC isolates (Figure 1). In a method comparison study, no false positives or false negatives were found (Nicola, Nievas & Smayevsky, 2012). However, they pointed out the need to adjust the distance between the discs to 12 mm center to center. In another study, Josa & cols. (2018) compared the PCR, boronic acid and immunochromatographic methods (with another kit), obtaining good results and a very low number of false negatives and false positives.

In a study in Venezuela, 3 of 22 strains of K. pneumoniae were negative for the boronic acid test. These 22 strains possessed the blaKPC gene, that is, with the capacity to produce carbapenemase. In that study, Martínez & cols. (2016) do not describe the distance between the disks and this could explain this 13.6% of false negatives.

On the other hand, Reyes-Chacón & cols. (2017) described a 5% false positive rate, comparing the boronic acid test with gene detection by PCR.

In the original description of the method (Doi & cols., 2008), the authors indicate a very good correlation with molecular detection by PCR, although they point out the limitation of the few samples studied.

Regarding the application of the Resist-3 O.O.K.® immunochromatographic test; Pasteran et al., 2016 reported a sensitivity and specificity of 100%, with positive and negative predictive values of 100%.

The application of methodologies different from the conventional ones represents a significant benefit in the clinical field, since it allows a reliable identification in a short period of time. Compared with molecular biology tests, immunochromatographic tests turn out to be cheaper and their routine application in microbiology laboratories improves the management of critical patients, reduces the use of broad-spectrum antibiotics and reduces hospital stays. In this work, the PCR test, considered the gold standard, was not performed. However, the use of both tests in the 26 positive cultures studied suggests that the results are reliable.

In this work we also study which variables can be associated with an increased risk of colonization by An important pillar of surveillance programs is the early detection of patients colonized by multiresistant pathogens, since it is a considerable risk factor for posteri. In the gender variable, the most affected patients were men with a predominance of age over 60 years. These results are consistent with different investigations, including Aguilar-Gamboa (2016), who determined that the most frequent patients with KPC were men with 68.4%; observing that 42.1% of them had an age range of 58-77 years.

The risk factors that were associated with rectal colonization by KPC were "prolonged hospitalization" and admission to the "Intensive Care Unit". It should be noted, however, that these two factors indicate a degree of severity in the patient's state of health that allows us to assume a high mortality rate. Indeed, numerous studies account for this (Carhuachagua Huarcaya & Pecho Torres, 2020).

In various studies, hospitalization time is mentioned as a variable associated with rectal colonization by KPC (Schwaber & cols., 2008; Paño Pardo & cols., 2014). These findings coincide with this study, where we found that 100% of colonized patients had a hospital stay > 25 days. This association has been described in the literature, in which colonization in long-term hospitalization centers is close to 30% in contrast to short-term hospitalization centers where it is 3% (Lin & cols., 2013). In this regard, colonization rates increase up to 3 times in the hospital environment in proportion to the time of hospitalization (Quispe & cols., 2018).

Hospitalization in the "Intensive Care Unit" as a risk factor has been reported in various publications (Schwaber & col., 2008) and is related to the severity of patients undergoing various invasive procedures (central venous access, nasogastric tube, ARMS, etc.). In this investigation, 100% of KPC carriers passed through the "Intensive Care Unit", acquiring this bacterium during their stay in that area. These high percentages may correspond to the fact that patients hospitalized in critical areas are those with impaired immune systems; due to the use of different invasive devices and the selective pressure exerted by the use of antibiotics, which translates into changes in the microbiota of each patient (Daikos & cols., 2012).

Other factors described in the literature are exposure to invasive procedures and antimicrobials. There are several articles that analyze the mentioned topic (Schwaber & cols., 2008; Lin & cols., 2013; Paño Pardo & cols., 2014). These authors point out that the use of antibiotics in previous instances was a relevant risk factor for colonization by KPC-producing K. pneumoniae. These antibiotics include third-generation cephalosporins, fluoroquinolones, aminoglycosides, ureidopenicillins, and carbapenems (Echeverri & cols., 2010). In disagreement, in this study the use of invasive devices and antibiotic treatment were not variables associated with colonization by KPC.

The risk factors analyzed in this work differ from those found by Saavedra et al. (2018), who found that surgery and previous antibiotic treatment were important risk factors for acquiring KPC.

Regarding white blood cell and hematocrit values, KPC-colonized patients had elevated white blood cell counts and low hematocrit. These values are usually present in infectious processes, so their association is to be expected and does not represent a particular biological meaning. We have not found any study that considered the association between the values of white blood cells and hematocrit and the risk of colonization by KPC.

The results of this study made it possible to establish the prevalence of CPE colonization in hospitalized patients and the lack of implementation of an adequate infection control program in the institution. Many authors consider that the application of infection control measures is key and requires that they contain aspects related to surveillance (early detection of the index case, active detection of colonization in patients), the implementation of standard and contact precautions, including cohorting measures, if necessary, in addition to environmental cleaning and disinfection (Carmeli & cols., 2010).

These procedures are consistent with the measures adopted by the institution, where, based on the results of the active surveillance cultures, from the Infection Control Committee and the Infectology Service of the Clinic, a series of preventive measures were implemented, such as the use of contact precautions for KPC patients, their hospitalization in individual rooms and/or cohorting, the restriction of the movement of personnel in critical areas such as the intensive care unit and the performance of weekly surveillance swabs in the therapy unit intensive and every 2 weeks in general hospital wards in order to detect new cases. This resulted in a strong decrease in patients colonized by KPC in the months after this study was carried out, where it fell to 2.9% of patients who were carriers of KPC (Ponce, unpublished data).

Compliance with control measures in accordance with international recommendations, as well as permanent epidemiological surveillance, are key factors for the prevention of infections

One limitation of this work is that the laboratory of the Cruz del Sur Clinic is of medium complexity and does not have the equipment or techniques for genetic analysis. There are also no laboratories with such technology at the local branch of the Universidad Nacional de la Patagonia Austral, so it was not possible to establish a possible clonal origin of the KPC strains.

Conclusions

The presence of KPC-type carbapenemase-producing K. pneumoniae was detected in rectal swab samples in 12.5% of the patients analyzed, out of a population of 208 patients. 77% of patients colonized by KPC progressed to infection and died during hospitalization.

Elevated white blood cell and low hematocrit values showed a significant association with rectal colonization by KPC. In addition, "prolonged hospitalization" and admission to the "Intensive Treatment Unit" were significantly related as risk factors for colonization by carbapenemase-producing K. pneumoniae.

There was no association between the pathologies of the patients analyzed and the presence of KPC. In summary, this work confirms the presence of KPC-type carbapenemase-producing K. pneumoniae in rectal swab samples in this Patagonian region of Argentina.

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