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Caracterización de Enterobacterias productoras de Carbapenemasas, a partir de aislados clínicos del Hospital Homero Castanier Crespo, Azogues-Ecuador

Characterization of Carbapenemase-producing Enterobacteriaceae, from clinical isolates from the Homero Castanier Crespo Hospital, Azogues-Ecuador

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Palabras claves:
Enterobacteriaceae; Antimicrobianos; Carbapenemasas; Resistencia bacteriana; Infección Hospitalaria

Resumen

Introducción. La resistencia a los antibióticos constituye un problema global cada vez más importante, el uso excesivo e irracional de antibióticos desencadena el desarrollo de cepas resistentes como las enterobacterias productoras de carbapenemasas (EPC), reduciendo las opciones de tratamiento disponibles en el campo clínico. Esta aparición de nuevas cepas resistentes a casi todos los agentes antimicrobianos es un resultado del uso inadecuado de los mismos, automedicación e incumplimiento terapéutico. Además del aumento de las tasas de resistencia a los carbapenémicos, durante la pandemia de COVID-19 se han demostrado emergencias con microorganismos altamente resistentes, lo que puede estar relacionado con un mayor uso de antibióticos de amplio espectro por parte de los pacientes. **Objetivo.** Caracterizar Enterobacterias productoras de carbapenemasas, a partir de su frecuencia en las áreas hospitalarias y tipo de muestra en pacientes que fueron ingresados al Hospital Homero Castanier Crespo (HHCC) de la ciudad de Azogues, Ecuador período enero 2020 hasta abril 2022. **Metodología.** Es un estudio de tipo positivista con un enfoque cuantitativo de corte transversal descriptivo, de tipo documental. **Resultados.** El 2021 fue el año con más incidencia de EPC 69,6%, el grupo etario más afectado fue el adulto (30-59 años) con un 54,9% y se presentó con mayor frecuencia en el género masculino 76,5%. La presencia de EPC fue más común en muestras de secreción endotraqueal del área de unidad de cuidados intensivos 70,1%, el agente etiológico con mayor incidencia fue *Klebsiella pneumoniae* 90,19%. Todos los aislados clínicos fueron resistentes a los betaláctamicos, los antibióticos con mejor sensibilidad 96% fueron gentamicina y amikacina **Conclusión.** Los resultados demuestran un incremento de infecciones por enterobacterias productoras de carbapenemasas y *Klebsiella pneumoniae* fue la especie bacteriana con mayor prevalencia. Entre estos aislados se determinó resistencia del 100% a los antibióticos betaláctamicos, incluidos los carbapenémicos que se consideran de última línea, lo que resalta la importancia de un manejo adecuado de la resistencia a los antimicrobianos. **Área de estudio:** bacteriología

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Keywords:
Enterobacteriaceae;
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Bacterial resistance;
Hospital Infection

Abstract

Introduction. Antibiotic resistance constitutes an increasingly important global problem. The excessive and irrational use of antibiotics triggers the development of resistant strains such as carbapenemase-producing Enterobacteriaceae (CPE), reducing the treatment options available in the clinical field. This emergence of new strains resistant to almost all antimicrobial agents is a result of their inappropriate use, self-medication, and therapeutic non-compliance. In addition to increasing rates of resistance to carbapenems, emergencies with highly resistant microorganisms have been demonstrated during the COVID-19 pandemic, which may be related to increased use of broad-spectrum antibiotics by patients. objective. Characterize carbapenemase-producing Enterobacteriaceae, based on their frequency in hospital areas and type of sample in patients who were admitted to the Homero Castanier Crespo Hospital (HHCC) in the city of Azogues, Ecuador, period January 2020 to April 2022. Methodology. It is a positivist study with a quantitative, cross-sectional, descriptive, documentary-type approach. Results. 2021 was the year with the highest incidence of CLD 69.6%, the most affected age group was adults (30-59 years) with 54.9% and it occurred most frequently in males 76.5%. The presence of CLD was more common in samples of endotracheal secretion from the intensive care unit area 70.1%, the etiological agent with the highest incidence was Klebsiella pneumoniae 90.19%. All clinical isolates were resistant to beta-lactams, the antibiotics with the best sensitivity 96% were gentamicin and amikacin Conclusion. The results demonstrate an increase in infections by carbapenemase-producing Enterobacteriaceae and Klebsiella pneumoniae was the most prevalent bacterial species. Among these isolates, 100% resistance to beta-lactam antibiotics was determined, including carbapenems that are considered last-line, which highlights the importance of adequate management of antimicrobial resistance.

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Introduction

Infections caused by carbapenemase-producing enterobacteria (CPE) have become a serious threat to health worldwide, especially at the hospital level.(1)These infections are difficult to treat and are associated with high morbidity and mortality due to: the few valid therapeutic options, their ability to spread and rapid colonization of patients.(23)The growing resistance to carbapenems is generated by the indiscriminate use of antibiotics, leaving doctors without therapeutic alternatives to effectively combat the infections produced and increasing the time of agony of patients, as well as the risk of death since they are considered last-line antibiotics in the treatment of these infections.(4).

The World Health Organization (WHO) has presented a list of the main bacteria that in recent years have shown higher levels of resistance to various antibiotics that put the health of the population at risk, including Klebsiella pneumoniae and Escherichia coli.(5,6).Resistance in enterobacteria is attributed to: different mobile genetic elements "plasmids and transposons" which allow it to move between bacterial genera or species, existing genetic mutations, as well as obtaining new resistance genes through conjugation, translation and transformation processes.(7,8).

EPCs can cause different types of infections, including urinary tract infections related to the use of urinary devices, respiratory infections due to microaspirations, and less frequently surgical site infections, catheter infections, or infections of other intravascular devices.(9)Symptoms depend on the location of the infection and may include fever, abdominal pain, cough, shortness of breath, pain when urinating, and urinary urgency.(10,11)In some cases, infections caused by EPC can be serious and potentially fatal, especially in patients with weakened immune systems or in hospitalized patients with other serious health problems.(12).

In October 2021, PAHO/WHO declared an epidemiological alert, mentioning: “During the COVID-19 pandemic, the emergence of extremely resistant microorganisms and an increase in the incidence of resistance to carbapenems have been documented, possibly related to the increased use of broad-spectrum antibiotics in patients with COVID-19.(13)In Asia, in a study of cases (COVID-19) and controls (non-COVID-19) of patients infected with carbapenemase-producing enterobacteria in 2020, the presence of Klebsiella pneumoniae (80.8%), Serratia marcescens (11%) and Enterobacter cloacae (4.1%) was identified in both groups. The most common bacteria were KPC 56.2%, OXA-48 26% and VIM 17.8%.(14)In Spain, infection by enterobacteria was associated with a 23.3% mortality rate at 30 days due to the ease of transmission, with Klebsiella pneumoniae being the most frequent enterobacteria (95.1%), followed by Enterobacter cloacae (4.9%).(15).

In Latin America, the prevalence of enterobacteria is worrying. In a study carried out at the Regional Hospital of Coronel Oviedo-Paraguay in 2019 in the intensive care unit, the presence of Klebsiella pneumoniae (14%) was found and when evaluating the production of enzymes, the production of ESBL-type enzymes was found (85.7%) of the samples and the production of carbapenemase-type enzymes (14.3%) of the samples.(16).

Subsequent studies carried out in Ecuador on clinical isolates of Klebsiella pneumoniae at the Monte Sinai Hospital in the city of Cuenca in the years 2018-2020 showed that the prevalence of ESBL was 27.7% and KPC-type carbapenemases 7.7% with a higher presence in the male sex.(17).In the described background, there is evidence of a large increase in infections by EPC that contribute to resistance to carbapenem antibiotics. Therefore, it is limited to health professionals with few therapeutic options against these microorganisms, prolonging hospital stays and significantly increasing costs for the public health system.

For this reason, it is pertinent to know the main species of carbapenemase-producing enterobacteria, their antimicrobial susceptibility profile, resistance profile, and their classification according to multidrug-resistant (MDR), extreme drug-resistant (XDR), and pan-drug-resistant (PDR). According to the above, the main objective of the research was to characterize carbapenemase-producing enterobacteria from their frequency in the hospital area and type of sample in patients who were admitted to the Homero Castanier Crespo Hospital in the city of Azogues, Ecuador from January 2020 to April 2022.

Methodology

This study is of a positivist type with a quantitative, descriptive, cross-sectional approach, of a documentary type. The sample consisted of 102 records of clinical isolates carrying carbapenemase-producing enterobacteria, these made up the entire sample with a total coverage sampling. This information was collected from secondary sources entered into the database of the microbiology department from different clinical services of the Homero Castanier Crespo Hospital in the city of Azogues-Ecuador from January 2020 to April 2022.

Inclusion Criteria

All clinical isolate records that present complete information (microorganism, hospital area, sample type, carbapenemase resistance mechanism) of patients who were admitted to HHCC services in the period January 2020 – April 2022.

Exclusion Criteria

Records of clinical isolates that do not have complete information, clinical isolates identified with resistance types different from those in the study.

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Processing, analysis, summary and presentation of information

The data were collected in a secondary record sheet in the Excel program, consisting of the following variables: age group; sex; biological year; hospital area; type of sample; bacterial species; carbapenemase production; antimicrobial susceptibility and resistance categorization. For the statistical analysis, a database was generated in the SPSS program, the analysis was performed using descriptive statistics and frequency analysis. For the presentation of the results, double-entry tables, cross tables were used and the graphs were represented by bar charts.

Ethical Aspects

This study is based on the ethical principles set forth in the Taiwan Declaration of Helsinki, which states that the data of the patients who obtained the research isolates will be protected and kept private, they will be kept strictly confidential, the rights of no patient will be violated, the names of patients will not be handled because a numerical system will be used in which they will be coded, said information will not be used for other purposes, it will not be publicly managed and no new research will be carried out, respecting its purpose.

Results

From January 2020 to April 2022, 102 (100%) clinical isolates of carbapenemase-producing enterobacteria were reported. The main species isolated in the study population was Klebsiella pneumoniae (90.19%) followed by Klebsiella oxytoca (4.9%) and Klebsiella sp. (1.96%) (See Chart 1).

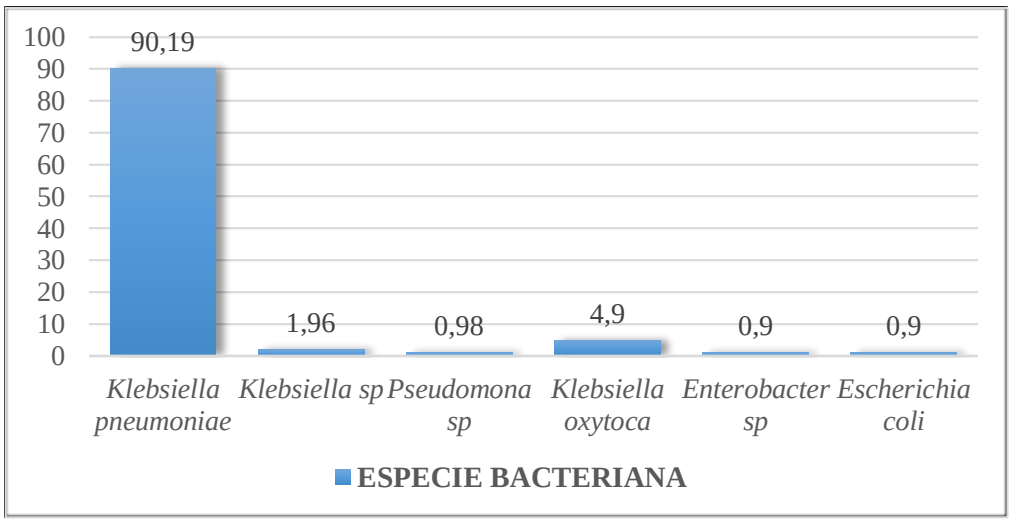

Chart 1. Percentage of major carbapenemase-producing Enterobacteriaceae present in clinical isolates from HHCC.

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The frequency of carbapenemase-producing Enterobacteriaceae per year was 29 (28.4%) clinical isolates in 2020, 71 (69.6%) in 2021, and 2 (2%) in 2022, which show significant differences. According to the results obtained, it can be observed that infections by carbapenemase-producing Enterobacteriaceae occur more frequently in adult patients (30-59 years), with a percentage of 54.9%, older adults (60 years or older) with a percentage of 36.2% and less frequently in young patients (18-29 years) with a percentage of 8.8% (See Chart 2).

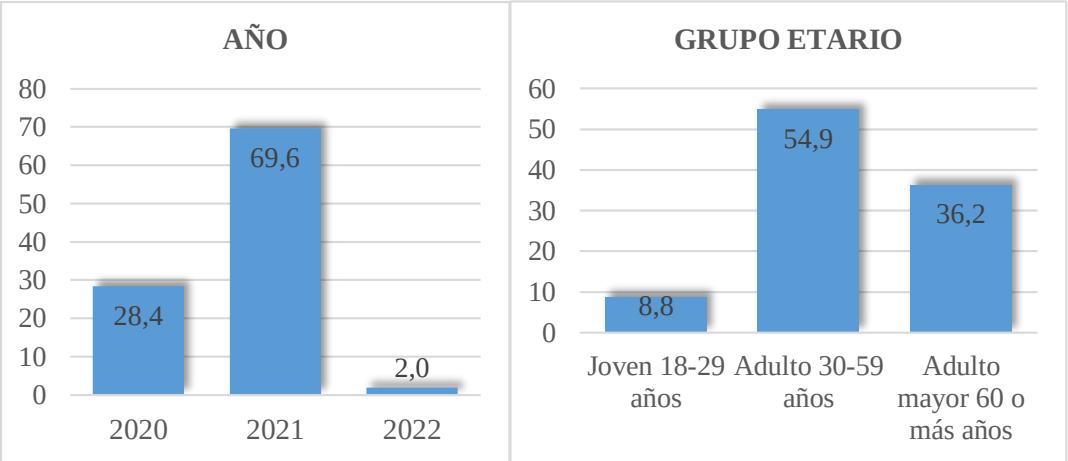

Chart 2. Frequency of carbapenemase-producing enterobacteria by year and age group present in clinical isolates from HHCC.

In this study, the gender distribution resulted in 78 clinical isolates (76.5%) belonging to the male gender and 24 (23.5%) to the female gender, showing significant differences (See Table 1).

Table 1. Gender prevalence of carbapenemase-producing Enterobacteriaceae present in clinical isolates from HHCC.

		GENDER			
		Frequency	Percentage	Valid percentage	Cumulative percentage
Valid	Male	78	76.5	76.5	76.5
	Female	24	23.5	23.5	100.0
	Total	102	100.0	100.0	

In relation to the hospital area and type of sample, the majority of positive clinical isolates come from the ICU (Intensive Care Unit) area 54 (70.1%) followed by the clinic 20 (26.0%), where the type of sample from which carbapenemase-producing enterobacteria were most frequently isolated was endotracheal secretion (See Table 2).

Table 2. Frequency of carbapenemase-producing enterobacteria present in clinical isolates from HHCC according to sample type and hospital area.

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		HOSPITAL AREA				
		ICU	Clinic	Surgery	Outpatient	Total
SAMPLE TYPE	Endotracheal secretion	70.1% (54)	26.0% (20)	2.6% (2)	1.3% (1)	100% (77)
	Urine	75.0% (3)	25.0% (1)	0.0% (0)	0.0% (0)	100% (4)
	Pleural fluid	0.0% (0)	100% (1)	0.0% (0)	0.0% (0)	100% (1)
	Sputum	50.0% (1)	50.0% (1)	0.0% (0)	0.0% (0)	100% (2)
	Blood	50.0% (3)	16.7% (1)	33.3% (2)	0.0% (0)	100% (6)
	Rectal	66.6% (4)	16.7% (1)	16.7% (1)	0.0% (0)	100% (6)
	Tissue	33.3% (1)	33.3% (1)	0.0% (0)	33,33(1)	100% (3)
	Pus	66.7% (2)	0.0% (0)	33.3% (1)	0.0% (0)	100% (3)
	Total	66.7% (68)	25.5% (26)	5.9% (6)	1,9(2)	100% (102)

The antimicrobial susceptibility profile of carbapenemase-producing enterobacteria species was also analyzed, as shown in Table 3, where 100% resistance to beta-lactam antibiotics from the penicillin family, third and fourth generation cephalosporins, monobactams, carbapenems and beta-lactamase inhibitors can be observed. The susceptibility profile of fosfomycin was also analyzed, where 75% of the cases presented sensitivity and 25% presented resistance to this antibiotic; while the quinolone family (ciprofloxacin) presented a sensitivity of 17.6%, intermediate 12.7% and resistance of 57.8%. Gentamicin and Amikacin remained as therapeutic options with a sensitivity of 96%.

Table 3. Antimicrobial susceptibility profile of carbapenemase-producing enterobacteria present in clinical isolates from HHCC.

ANTIBIOTIC	Sensitive %	Intermediate %	esistant %
Amoxicillin Clavulanic acid	0.0 (0)	0.0 (0)	100.0 (102)
Ampicillin Sulbactam	0.0 (0)	0.0 (0)	100.0 (102)
Piperacillin Tazobactam	0.0 (0)	0.0 (0)	100.0 (102)
Ceftazidime	0.0 (0)	0.0 (0)	100.0 (102)
Cefotaxime	0.0 (0)	0.0 (0)	100.0 (102)

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Ceftriaxone	0.0 (0)	0.0 (0)	100.0 (102)
Cefepime	0.0 (0)	0.0 (0)	100.0 (102)
Imipenem	0.0 (0)	1.0 (1)	99.0 (101)
Meropenem	0.0 (0)	0.0 (0)	100.0 (102)
Aztreonam	0.0 (0)	0.0 (0)	100.0 (102)
Gentamicin	96.1 (98)	0.0 (0)	3.9 (4)
Amikacin	96.1 (98)	0.0 (0)	3.9 (4)
Ciprofloxacin	17.6 (18)	12.7 (13)	57.8 (59)
Fosfomycin	75.0 (3)	0.0 (0)	25.0 (1)

Resistance categorization was not performed according to the guidelines established by Re LAVRA 2019 based on the classification criteria for multidrug-resistant (MDR), extreme drug-resistant (XDR), pan-drug-resistant (PDR) because the antibiotics Trimethoprim-sulfamethoxazole, tigecycline, and colistin were not tested.

Discussion

The increasing frequency of carbapenemase-producing enterobacteria that block carbapenem antibiotics crucial in the treatment of serious infections mainly in hospital settings, represents a major challenge for public health in terms of morbidity, mortality and healthcare costs due to the limited treatment options available in the clinical field to treat these infections.(18); therefore, the World Health Organization has categorized carbapenem-resistant Enterobacteriaceae as a critical priority (Priority 1)(19).

In the present study, from January 2020 to April 2022, 102 positive clinical isolates of EPC were reported, from hospital areas of the HHCC. The cases of EPC present in clinical isolates increased significantly in 2020 (28.4%) and 2021 (69.6%) this could be justified by the COVID-19 pandemic because when cases in hospitals increased, so did the use of antibiotics, as described by the CDC (Centers for Disease Control and Prevention) in the 2022 report.(20)**;**It is important to note that EPCs are common in critically ill and immunocompromised patients.(21), in this sense, a large part of the positive clinical isolates in our research came from the Intensive Care Unit area (70.1%) followed by the Clinic (26.0%). Epidemiological data reveal a considerable increase in EPC infections that are often isolated from clinical samples such as urine in urinary tract infections, blood in bacteremia, as well as respiratory in cases of pneumonia.(22)which differs from this investigation in which a greater presence of EPC was presented in samples of endotracheal secretion, which suggests a dissemination and a probable colonization of the germ in the ICU area where the most frequent cause of this transmission is the transfer of plasmids that leads to the colonization of these hospital services, as well as to prolonged treatments with broad-spectrum antibiotics that they received during their hospital stay, a

conjecture that is consistent with that of various studies where they refer to colonization by EPC in ICU and other hospital services.(16).

The predominant species in this study was Klebsiella pneumoniae (90.19%) which coincides with what was reported by Pintado et al in Asia in a study of cases (COVID-19) and controls (non-COVID-19) of patients infected with EPC in 2020, along the same lines Antequera et al.(15)In their study carried out in Spain, they identified that EPC infection was associated with a 23.3% mortality rate at 30 days due to the ease of transmission and Klebsiella pneumoniae was the most frequent etiological agent (95.1%). The incidence of this species as the main causal agent of infections acquired in hospitals and even in the community has had a substantial increase in recent decades, associated with its resistance patterns.(23,24)These studies confirm that our country is facing the same problem that is reflected worldwide. The highest number of clinical isolates of this species occurred in the group of adults between 30-59 years of age, which verifies the capacity of this microorganism to infect immunosuppressed individuals.(25)closely related to patients infected by Covid-19. In the present investigation, the male gender was more susceptible to contracting these infections, which can be attributed to multiple clinical pathologies such as complicated urinary tract infections, weakness of the immune system and, mainly, the inappropriate use of antibiotics, which differs from previous studies carried out by Álvarez et al.,(17)or Guaraca et al.,(26)in which the female gender was more susceptible to these infections.

The high presence of resistance mechanisms such as carbapenemases is worrying, as various national and international studies have shown(3,14,15)They demonstrate their ability to hydrolyze most of the beta-lactam antibiotics, which are considered last-line antibiotics. In this study, all clinical isolates according to the antimicrobial susceptibility profile presented 100% resistance to beta-lactam antibiotics, including carbapenems. This finding is consistent with what has been expressed previously. However, gentamicin and amikacin, which are considered therapeutic alternatives, presented 96% sensitivity, remaining as valid therapeutic options in the present study. These antibiotics should be used in combined regimens even though studies show that the risk of toxicity has been present.(27)It is important to mention that 4% of the clinical isolates were resistant to gentamicin and amikacin, reducing therapeutic options to almost zero, a condition that demonstrates the vulnerability of patients and the importance of knowledge of the personnel working in medical care, especially in the ICU, on antimicrobial management strategies, which will increase the possibility of therapeutic success.

Conclusions

- In the health field, the increase in infections by carbapenemase-producing enterobacteria is worrying because it limits the treatment options available in the clinical setting and increases the severity of the infection and mortality. In the

population under study, cases increased significantly during the Covid-19 pandemic in 2020 and 2021, which shows that these patients have a higher risk of infections due to highly resistant EPC, which usually appear in critically ill patients. Klebsiella pneumoniae was the most frequent etiological agent. The majority of positive clinical isolates corresponded to the intensive care unit area in endotracheal secretion samples, suggesting bacterial dissemination and possible colonization of this area. The most common cause of this transmission is the acquisition of new resistance genes by horizontal transfer. Susceptibility results demonstrated resistance to antibiotics.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution statement

Author 1: Data processing, writing and synthesis of the manuscript

Author 2: Application of statistical analysis, review and final validation of the manuscript

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