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GENOTYPIC CHARACTERIZATION OF K. PNEUMONIAE AND E. COLI CARBAPENEMASES ISOLATED FROM PEDIATRIC SAMPLES AT THE HOSPITAL GENERAL DE ENFERMEDADES

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Abstract: Antibiotic resistance is a global problem. Among the priority pathogens defined by the World Health Organization (WHO) are enterobacteria resistant to carbapenems (CRE), which increase hospitalization time, cost and mortality and reduce therapeutic options. The early detection of the type of Carbapenemase allows guiding therapeutic approach, knowing epidemiology, controlling intra-hospital infections and avoiding outbreaks. The objective was to genotypically characterize CRE carbapenemases from pediatric samples from a Third Level Hospital in Guatemala. The study was descriptive, observational, cross-sectional. Of the patients admitted to the Pediatric area from 12/01/2019 to 12/31/2020, the E.coli and K.pneumoniae strains whose MIC showed resistance to imipenem and/or ertapenem were analyzed. Genotypic identification was performed by amplification of the target genes (blaKPC, blaVIM, blaNDM, blaOXA-48 and blaIMP gene) by real-time polymerase chain reaction (PCR) (GeneXpert® Carba-R). The data was processed with SPSS. 62 strains of K. pneumoniae and 24 strains of E. coli resistant to carbapenems were analyzed. 96.77% and 95.83% respectively presented at leastone type of gene evaluated, the most prevalent was blaNDM (59/60, 98.33% and 22/23, 95.65% respectively). One strain of K. pneumoniae presented blaKPC-type carbapenemase and one strain of E. coli presented blaNDM and blaKPC. No genes for VIM, IMP, or OXA-48 carbapenemases were found. The service with the most isolations was the Intensive Care Unit. More than 33% of CRE were recovered from urine cultures. The genotypic characterization evidenced the presence of the blaNDM gene in more than 95 % of the CRE isolated from pediatric samples from a Third Level Hospital in Guatemala.

Keywords: Carbapenemase, gene, antibiotic resistance.

INTRODUCTION

The Global Surveillance System for Antimicrobial Resistance(GLASS), has reported the presence of antibiotic resistance in samples of 500,000 people from 22 countries in which bacterial infections were suspected. (WHO, 2018). Among the main pathogens that can present alarming resistance are Carbapenem-resistant Enterobacteriaceae (WHO, 2017).

Resistance to carbapenems can occur by various mechanisms, the main one being the production of enzymes.carbapenemases. The relevance of carbapenemases lies in their ability to hydrolyze even the latest alternatives of current antibiotics, limiting therapeutic options.(Hammoudi & Ayoub, 2020). Its wide geographical dissemination is of importance since they are found both in clinical isolates and in environmental and zoonotic bacteria, thus becoming a threat to health (Codjoe & Donkor, 2017; Hammoudi & Ayoub, 2020).

In Guatemala it was reporteda resistance for K. pneumoniae of 28.83% against imipenem(PAHO/WHO, 2019)during 2016, with NDM being the most prevalent carbapenemase, phenotypically and genotypically (Garrido, 2014; Velásquez, 2016; Chinchilla, Tomas, & Morales, 2013), while for E.coli in Latin America it was 9% (Nordmann & Poirel, 2019), with KPC being the most prevalent enzyme, followed by NDM (Chinchilla, Tomas & Morales, 2013).

In this investigation, all K.pneumoniae and E.coli were studied, from pediatric samples from a Third Level Hospital in Guatemala, which showed high minimum inhibitory concentration (MIC) to carbapenems in the evaluation of initial sensitivity. Only the first strain per type of sample per patient was included to avoid duplication of data. The characterization was carried out with the gold standard: molecular method. Genotypic identification was performed by amplifying

the target genes (blaKPC, blaVIM, blaNDM, blaOXA-48 and blaIMP gene) through a real-time polymerase chain reaction (PCR) (GeneXpert® Carba-R).

BACKGROUND

ANTIBIOTIC RESISTANCE

Antibiotic resistance is one of the biggest threats to global health today.(De la Lastra, Ulloa, Pinto, Vidal, & Silva, 2010; WHO, 2020). GLASS proposes to support surveillance and research at the global level to strengthen the evidence of antimicrobial resistance and help decision-making at the national, regional and global levels. GLASS has revealed the widespread presence of antibiotic resistance in samples of 500,000 people from 22 countries(Biomerieux, 2020;Nordmann & Poirel, 2019; World Health Organization, 2018).

In Latin America, the Latin American Network for Antimicrobial Resistance Surveillance (ReLAVRA) raises antimicrobial resistance as an urgent public health priority. (PAHO/WHO, 2019). It is intended to respond to resistance based on laboratory surveillance, monitoring and implementation of awareness campaigns and development of antimicrobial administration programs in hospitals.(Jeon, et al., 2015).

According to GLASS, the most frequent resistant bacteria are Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus and Streptococcus pneumoniae, followed by Salmonella spp.(Nordmann & Poirel, 2019; WHO, 2018). During 2017, on the WHO list of priority pathogens, Carbapenem-Resistant Enterobacteriaceae (CRE) were found, which have become a global problem.

Recognizing the risks of infection by bacteria resistant to carbapenems, especially in vulnerable patients, as well as early detection of the specific resistance mechanism, are critical to reduce the risk of mortality, hospitalization time and associated costs.(Nordmann & Poirel, 2019).

CARBAPENEMICS

Carbapenems are potent antibiotics of the family ofb-lactams. Generally, they are the antibiotics of choice for invasive infections or those that compromise the patient's life due to their broad spectrum (Codjoe & Donkor, 2017).

They have high affinity for PBP enzymes (penicillin-binding proteins), involved in the assembly of the peptidoglycan structure, vital for the formation of the bacterial cell wall (Moreno, 2013).

In bacteriaGram negative carbapenems first cross the porins of the outer membrane and bind to the serine residues of the PBPs (especially high molecular weight PBPs), inhibiting the synthesis of the bacterial cell wall during transpeptidation, preventing it from spreading. assemble correctly, resulting in its weakening and finally bacterial lysis (Moreno, 2013).

RESISTANCE TO CARBAPENEMICS

Enterobacteriaceae can present resistance to carbapenems by three mechanisms: efflux pump, loss or mutation of porins, and production of carbapenemases (main mechanism).(Hammoudi & Ayoub, 2020; Wei, Yang, Ye & Li, 2015).

Resistance can be intrinsic or acquired.It is intrinsic when bacteria, both commensal and pathogenic, tend to be naturally resistant, which limits and complicates the selection of antibiotics for treatment, thus ending in a high risk of developing acquired resistance. On the other hand, acquired resistance is mediated by transferable genes, which can be dispersed among different bacterial genera (Codjoe & Donkor, 2017).

EPIDEMIOLOGY ACCORDING TO THE BACTERIA STUDIED

- K. pneumoniae

The world report on surveillance reported that during 2014 *K. pneumoniae* presented resistance to carbapenems in proportions that even reached 54%(World Health Organization, 2014).According to the Pan American Health Organization, according to the Antimicrobial Resistance Surveillance Network (ReLAVRA), the non-sensitivity to imipenem (intermediate and resistant resistance) of K. pneumoniae during 2014 was 23.33%, in in 2015 of 55.02% and during 2016 of 57.18% (last updated data in the PAHO network). During 2016, resistance to imipenem specifically in Guatemala was 28.83% (PAHO/WHO, 2019).

In relation to the type of carbapenemases, phenotypically it has been reported that in Guatemala 93% MBL (Garrido, 2014). Genotypically the most common is NDM, in proportions of 85 to 91%, followed by KPC (15%) (Velásquez, 2016; Chinchilla, Tomas, & Morales, 2013).

- E.coli

Up to 9% resistance to carbapenems has been reported in Latin America. Regarding the types of carbapenemases described, the CDC (Center for Disease Control and Prevention) reported that in the United States of America, 47.9% were KPC producers, although other carbapenemases (NDM, VIM, and OXA-48) have also been reported. documented. In Canada, the production of KPC (66.9%) and NDM-1 (17.3%) has been reported annually (Nordmann & Poirel, 2019).

Specifically in Guatemala, the study carried out by Chinchilla, Tomas & Morales (2013) reported that the most prevalent enzyme was KPC (63%), followed by NDM (37%).

DIAGNOSIS OF CARBAPENEMS

Rapid detection is necessary to prevent its spread and the appearance of Nosocomial infections, for epidemiological surveillance and screening of patients during an outbreak. (Biomerieux, 2020; Wei, Yang, Ye & Li, 2015).

SUSPICION AND SCREENING TESTS

Mean inhibitory concentration (MIC): An intermediate or high MIC raises the suspicion of the presence of carbapenemases (Wei, Yang, Ye & Li, 2015).

Phenotypic screening tests: Phenotypic evaluation does not allow for reliable identification, but they are useful for initial screening (disc diffusion, E-test or others) (Biomerieux, 2020).

MOLECULAR TESTS

Based on the polymerase chain reaction (PCR), sometimes followed by sequencing, they are the gold standard. Advantages include speed, excellent sensitivity and specificity. The disadvantage is the need for trained personnel, the inability to detect new genes involved, and the cost (Velásquez, 2016).

GeneXpert ® Carba-R:

It is real-time PCR, test*in viro*, qualitative that identifies the sequences of the blaKPC,, blaVIM, blaNDM, blaOXA-48 and blaIMP genes. One cartridge is used per sample and is processed in the corresponding equipment, fully automated(Cepheid, 2013).

OBJECTIVES

GENERAL OBJECTIVE

- Genotypically characterize K.pneumoniae and E.coli carbapenemases isolated from pediatric samples at a General Disease Hospital.

SPECIFIC OBJECTIVES

- To determine the most prevalent type of carbapenemase in a Third Level

Hospital in Guatemala in the isolates from the Department of Pediatrics for K.pneumoniae and E.coli bacteria.

- Characterize the type of sample in which the largest number of carbapenemase-producing K. pneumoniae strains were isolated.
- Characterize the type of sample in which the largest number of E.coli strains that produce carbapenemases were isolated.
- Identify the service with the highest prevalence of carbapenemase-producing K. pneumoniae.
- Identify the service with the highest prevalence of carbapenemase-producing E.coli.

METHODOLOGY

The present study was observational, cross-sectional. The data analyzed will correspond to the analysis of the dates from 12-01-2019 to 12-31-2020.

Initially, K.pneumoniae and E.coli bacteria from any type of sample from the Department of Pediatrics were evaluated for their sensitivity in the Vitek 2 equipment, by means of the Minimum Inhibitory Concentration (MIC). Those who presented resistance to carbapenems (resistance to ertapenem and intermediate or resistant to imipenem, evidenced by a high MIC), were included in the study. One strain per type of sample per patient was analyzed in order to avoid duplication.

PCR (polymerase chain reaction) was performed using GeneXpert ®Carba-R cartridges in the corresponding equipment. Thus, the following types of carbapenemases were screened: NDM, OXA, KPC, VIM, IMP.

Finally, the data were processed in the statistical program SPSS.

SCOPE

The study characterized the types of carbapenemases in strains of K.pneumoniae and E.coli from any type of sample, with resistance to carbapenems evidenced by high MIC, from the Pediatrics at a Third Level Hospital in Guatemala.

LIMITATIONS

Although the production of carbapenemase enzymes is the main mechanism for the presence of resistance to carbapenems, there are others that could not be characterized (efflux pumps and porin mutations). Another limitation is that only five types of carbapenemases, available in the commercial kit, were evaluated.

ETHICAL ASPECTS

The personal data of the patients were safeguarded. With the data obtained, decision-making can be supported.

RESULTS

resistance mechanism	KPN	ECHO
	% (n)	% (n)
Carbapenemase production	96.77 (60/62)	95.83 (23/24)
No production of carbapenemase*	3.23 (2/62)	4.16 (1/24)

KPN= K.pneumoniae, ECO= E.coli, * Carbapenemases evaluated (OXA-48, NDM, KPC, VIM, IMP).

Table No.1 Resistance mechanism of K. pneumoniae and E. coli isolated from pediatric samples

Source: Data obtained experimentally.

Table No. 1: it is observed that the main mechanism of resistance of K.pneumoniae and E.coli was the production of at least one of the carbapenemases evaluated (96.77% and 95.83% respectively).

Carbapenemase characterization	KPN	ECHO
	% (n)	% (n)
NDM	93.33 (59/60)	95.65 (22/23)
KPC	1.67 (1/60)	0.00 (0/23)
VIM	0.00 (0/60)	0.00 (0/23)
OXA-48	0.00 (0/60)	0.00 (0/23)
IMP	0.00 (0/60)	0.00 (0/23)
NDM + KPC	0.00 (0/60)	4.35 (1/23)

KPN= K.pneumoniae, ECO= E.coli

Table No. 2 Genotypic characterization of carbapenemases produced by K.pneumoniae and E.coli isolated from pediatric samples

Source: Data obtained experimentally.

Table No. 2: it is evident that in most of the strains that produce carbapenemases, both K. pneumoniae and E. coli, the blaNDM gene was detected (93.33% and 95.65% respectively). Regarding K. pneumoniae, only one of the strains carried the blaKPC gene. In one of the E.coli strains, both the blaNDM gene and the blaKPC gene were detected.

Service	KPN	ECHO
	% (n)	% (n)
Pediatric Intensive Care Unit	41.67 (25/60)	34.78 (8/23)°
Pediatric Intermediate Care Unit	13.33 (8/60)*	30.43 (7/23)
Pediatric Emergency	23.33 (14/60)	8.70 (2/23)
General Pediatrics	8.33 (5/60)	13.04 (3/23)
Pediatric Specialties	10.00 (6/60)	8.70 (2/23)
Pediatric infectious disease	1.67 (1/60)	4.35 (1/23)
Pediatrics Covid Area	1.67 (1/60)	0.00 (0/23)

KPN= K.pneumoniae, ECO = E.coli, ° Strain of ECO producing NDM and KPC * Strain of KPN producing KPC

Table No.3 Services from which samples from pediatric patients with carbapenemase-producing strains of K.pneumoniae and E.coli came from

Source: Data obtained experimentally.

Table No. 3 shows that the hospital service with the highest number of carbapenemase-producing strains (K.pneumoniae and E.coli) was the Pediatric Intensive Care Unit (41.67% and 34.78% respectively).

Sample types	KPN	ECHO
	% (n)	% (n)
Urine	33.33 (20/60)	52.17 (12/23)°
Blood	26.66 (16/60)*	13.04 (3/23)
Secretion	25.00 (15/60)	30.44 (7/23)
Liquid	0.00 (0/60)	4.35 (1/23)
orotracheal aspirate	8.33 (5/60)	0.00 (0/23)
Operative wound	1.67 (1/60)	0.00 (0/23)
Sputum	1.67 (1/60)	0.00 (0/23)
Bone marrow	1.67 (1/60)	0.00 (0/23)
Catheter	1.67 (1/60)	0.00 (0/23)

KPN= K.pneumoniae, ECO = E.coli, ° Strain of ECO producing NDM and KPC * Strain of KPN producing KPC

Table No.4 Types of samples with K.pneumoniae and E.coli strains that produce carbapenemases

Source: Data obtained experimentally.

In Table No. 4 it is identified that, in the urine cultures, the greatest number of K. pneumoniae and E. coli strains that produce carbapenemases were isolated (more than a third, 33.33% and 52.17% respectively). Subsequently, K. pneumoniae was isolated from blood cultures (26.66 %) while E. coli was obtained more in secretion cultures (30.44 %).

DISCUSSION OF RESULTS

Most of the resistance to carbapenems for both K.pneumoniae and E.coli from pediatric samples was conferred by the production of carbapenemases (96.77% and 95.83% respectively) (Table 1). It has been reported that for the genus Enterobacteriaceae the main mechanism of resistance to carbapenems is

the production of easily transferable plasmid-encoded enzymes (Hammoudi & Ayoub, 2020).

In 3.23 % of K. pneumoniae and in 4.16 % of E. coli, the presence of carbapenemases was not detected, therefore resistance to carbapenems could have been mediated by different mechanisms such as porin mutation or efflux pump (Wei, Yang, Ye & Li, 2015). It must be noted that since the PCR analysis carried out contained the target genes of the five most common types of carbapenemases (blaKPC, blaVIM, blaNDM, blaOXA-48 and blaIMP gene), resistance due to the production of some different carbapenemase was not ruled out.

In relation to K. pneumoniae (Table 2), the considerable colonization efficiency that it possesses (in the nasopharynx and gastrointestinal tract of humans) together with the antibiotic resistance that it is capable of acquiring because it is a "plasmid collector", make it a bacterium. easily persistent and spreadable, especially in hospital settings (Martin & Bachman, 2018; Tzouvelekis, Markogiannakis, Psichogiou, Tassios, & Daikos, 2012). In the present study, it was notorious that the majority of the EPCs analyzed were from this bacterial species (72.29%), followed by E.coli (21.71%), the first opportunistic pathogen that is acquired both nosocomially and community-based (Gauthier et.al, 2018).

When carrying out the genotypic characterization of the carbapenemases of the K. pneumoniae strains (Table No. 3), it was obtained that 98.33% (59/60) were producers of New Delhi Metallobetalactamase (NDM), followed by KPC type serinecarbapenemase. (1.67%). This coincides with the national study by Garrido (2014), in which it was reported that 93% of the bacteria analyzed had MBL. Likewise, it agrees with other investigations from Guatemala in which it has been reported that the most common carbapenemase has been NDM, in proportions of 73 to even 100%, followed by KPC (Chinchilla, Tomas, & Morales, 2013; Guerra, Valenzuela, & Velásquez, 2020; Velásquez, 2016).

Regarding E.coli, in the country it was reported that during 2011 (Garrido,2014) none of the EPC analyzed were E.coli, however during 2013 (Chinchilla,Tomas & Morales) the appearance of carbapenemase-producing E.coli strains was evidenced as 65.85 % of the EPC evaluated were E.coli (63 % KPC-producing and 37 % NDM-producing). Next, Guerra and collaborators (2020), reported that in a Fourth Level Hospital, during 2014 of the analyzed CLD 12 % were E.coli while in 2015 it increased to 18%, being most of them NDM type carbapenemases producers (100% and 67% respectively). The latter findings correlated with those found in the present study, which identified that 27.71% of the CLDs were E.coli and 95.65% (22/23) were NDM producers. The concordance of the elevation of the prevalence percentage with the study by Guerra et al (2020) could be due to the fact that in both studies all the CLD strains from the hospital (in our case from a Third Level hospital) were evaluated, unlike the study by Chinchilla et.al (2013) in which only the samples referred from some private hospitals and others stored in the National Health Laboratory were analyzed.

The increase in the frequency of NDM-producing E.coli isolates was due to two factors: bacterial and human. The spread of the strains could have been favored by the bacterial versatility for horizontal (lateral) transfer of plasmids (the main form of transmission of carbapenemase-encoding genes, including the blaNDM gene) between strains, genera and species, coupled with the their heterogeneity in terms of their size and associated resistance genes (quinolones, aminoglycosides, among others), since there

are different types of plasmids that encode this gene, the predominant ones in America, for humans, being IncF and IncI (Johnson, 2013; Rozwandowicz et.al, 2018; Velásquez, 2016). Besides,in relation to the human factors that have favored the increase of NDM-producing E.coli are travel, food production and preparation, among others, since it has been found that patients colonized in the intestine with NDM-producing bacteria still seven months after their discharge, they continue to excrete these strains through urine and feces, for which reason the NDM-producing E.coli could be acquired in the community (Johnson, 2013; Wei, Yang, Ye & Li, 2015).

However, as regards the strain of E.coli (4.35%) carrying both the bla geneNDM and blaKPC, what was found correlated with what was reported by Chinchilla et.al (2013), in Guatemala, who observed the presence of both carbapenemases in this bacterial species in 5.08 %.

On the other hand, when analyzing the services from which the samples came, it was notorious that the majority (both K. pneumoniae and E. coli) were from the Pediatric Intensive Care Unit (Table 4), as was the case. This is to be expected, since in the hospital environments where carbapenems are most used (Intensive Care Unit and Intermediate Care Unit), carbapenemase-producing bacteria have a selective advantage over those that do not. Likewise, the transfer of plasmids is facilitated due to the existing reservoirs, such as colonized or infected individuals and medical supplies used (Velásquez, 2016).

Regarding the type of sample, the main culture with the presence of the carbapenemase-producing Enterobacteriaceae studied was urine culture (33.33% K.pneumoniae and 52.17% E.coli, Table 4). Regarding E.coli, it must be noted that it is responsible for up to 80% of urinary infections acquired in the community and that in the presence of ESBL-producing bacteria, the next line of treatment is carbapenems, which is why the main The source of carbapenem-producing E.coli was urine (Candan & Aksöz, 2017), followed by secretion cultures. These data agree with those reported in the country for this bacterium (Chinchilla, Tomas, & Morales, 2013).

Regarding carbapenemase-producing K. pneumoniae, the results coincided with reports that this bacterium is mainly found causing urinary infections (Martin & Bachman, 2018). Likewise, the second site where it was found to produce infection was in the blood, a finding that correlates with the literature since it is a common cause of infections such as pneumonia, urinary tract infections, and sepsis (Velásquez, 2016).

CONCLUSIONS

- The genotypic characterization of the types of carbapenemases for the studied bacteria isolated from pediatric samples from the Hospital General de Enfermedades, identified 98.33% of K. pneumoniae producing NDM and 1.33% producing KPC, while 95.65% of E.coli produced NDM and 4.35% produced NDM and KPC.
- The main mechanism of resistance to carbapenems in both K. pneumoniae and E. coli was the production of the evaluated carbapenemases (96.77 % and 95.83 % respectively).
- The main type of pediatric sample from which carbapenemase-producing strains were recovered was urine, with more than a third of the isolates for both bacteria (33.33% K. pneumoniae and 52.17% E.coli).
- The pediatric hospital service with the highest number of carbapenemase-producing strains was the Pediatric Intensive Care Unit.

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