Resistome analysis in *Enterobacter cloacae* genomes by in silico approach

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1 INTRODUCTION

The genus *Enterobacter* is composed of highly heterogeneous bacteria of Gram-negative bacilli, facultative anaerobes, rod-shaped, motile and spore-forming, being part of the family *Enterobacteriaceae*. In addition, they are characterized as ubiquitous microorganisms, where they can be found in varied environments, such as the gastrointestinal tract of humans and other mammals, hospital environments, wastewater, soil and water sources, and are also endophytic or phytopathogenic for several plant species (Murray, Rosenthal, Pfaller, 2014; Procop et al., 2018; Davin-Regli, Lavigne, Pagès, 2019).

The genus has 22 species, however, the species *E. cloacae, E. aerogenes* and *E. hormaechei* have clinical importance because they are related to infections triggered by *Enterobacter*. In this sense, the genus presents easy dissemination among humans, with this, they are calling for being pathogens related to hospital infections, and mainly, in patients with depressed immune system, giving rise to respiratory, urinary and gastrointestinal infections (Wang et al., 2017; Davin-Regli, Lavigne, Pagès, 2019).

Enterobacter cloacae has stood out as one of the main opportunistic and multidrug-resistant bacterial pathogens reported in hospitals in the last three decades, mainly in immunosuppressed patients in intensive care units (ICU). The agent can cause several infections, including septicemia, pneumonia, bacteremia, endocarditis, osteomyelitis, skin infections, septic arthritis. Among the signs and symptoms most commonly found in infections caused by the pathogen are fever, hypotension, shock, leukocytosis and systemic inflammatory response (Cai, Chen and Zhao, 2019; Ramirez; Giron, 2020). It is important to note that genomic studies have shown that the *Enterobacter cloacae* complex has the ability to acquire genes associated with resistance to broad-spectrum antibiotics, among the

genes, carbapenemase-related genes with intrinsic β-lactam resistance originating from chromosomal ampC genes stand out (Annavajhala, Gomez-Simmonds & Uhlemann, 2019).

Thus, a set of genes responsible for antimicrobial resistance through gene modifications, called the resistome, may be present in pathogenic bacteria, bacteria from the microbiota of animals and humans, environments such as soil, sewage, hospital waste and various water sources. In the past, increasing resistance among enterobacteria has been mainly due to the production of extended-spectrum beta-lactamases (ESBL), a mechanism mainly associated with *E. aerogenes* and *E. cloacae* species, responsible for resistance. *cloacae species,* responsible for resistance against penicillins, third- and fourth-generation cephalosporins and aztreonam (Davin-Regli; Pagès, 2015; López-Velandia, Torres-Caycedo; Prada-Quiroga, 2015; Davin-Regli, Lavigne, Pagès, 2019).

Thus, the growing increase in enterobacteria associated with infections, especially in the hospital environment, has become a serious public health problem due to the high levels of resistance in pathogens. However, most resistance studies in this family remain focused on the traditionally more prevalent genera, such as *Klebsiella spp.* even with the increase in cases of nosocomial infections triggered by species of the genus Enterobacter spp. mainly *Enterobacter cloacae.* Thus, the analysis of resistance genes will allow a greater knowledge about the epidemiology of this pathogen, in addition to contributing to the elaboration of preventive measures for the dissemination of the bacterium and its resistance genes, as well as assisting in the adoption of appropriate antimicrobial therapies.

2 OBJECTIVE

Analyze *in silico* the antibiotic resistance genes present in complete genomes of *Enterobacter cloacae.*

3 METHODOLOGY

Data collection

Thirty-one complete genomes of *Enterobacter cloacae from* the NCBI (National Center of Biotechnology) database were used, including strain-specific data such as isolation site and country of origin (Supplementary material).

Phylogenetic analysis

In the phylogenetic analysis, the 16s ribosomal gene of the E. cloacae genomes was used, in the multiple alignment the BioEdit program (Alzohairy, 2011) was used, through the ClustalW program. Then, the MEGAX program (Kumar et al, 2018) was used to build the phylogenetic tree, using the Maximum Parsimony method with 1000 bootstrap replicates as parameters. As outgroup for this analysis, the complete genomes of *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae*

HS11286 available in the NCBI database under accession number CP009072 and GCA_001086625.1 were used.

Islands of resistance

The identification of GIs (genomic islands) was performed using the Gipsy program (SOARES et al., 2016), which is responsible for the prediction of genomic characteristics shared between a pathogenic and a non-pathogenic species. In this analysis, embl format files of *E. cloacae* samples (query) were used as input along with a non-pathogenic strain, *Enterobacter oligotrophica* (subject) (Akita et al. 2019), as reference genome. The default parameters of the program for GI prediction were adopted: Gc content deviation 1.5, codon usage deviation 0.95, transposase genes 1E-04, search for specific factors 1E-06, tRNA genes 1E-04 and absence in other organisms 1E-06. In addition, for the analysis of the present work, among the genomic islands identified by the Gipsy program, the resistance islands were selected.

Analysis of antibiotic resistance genes

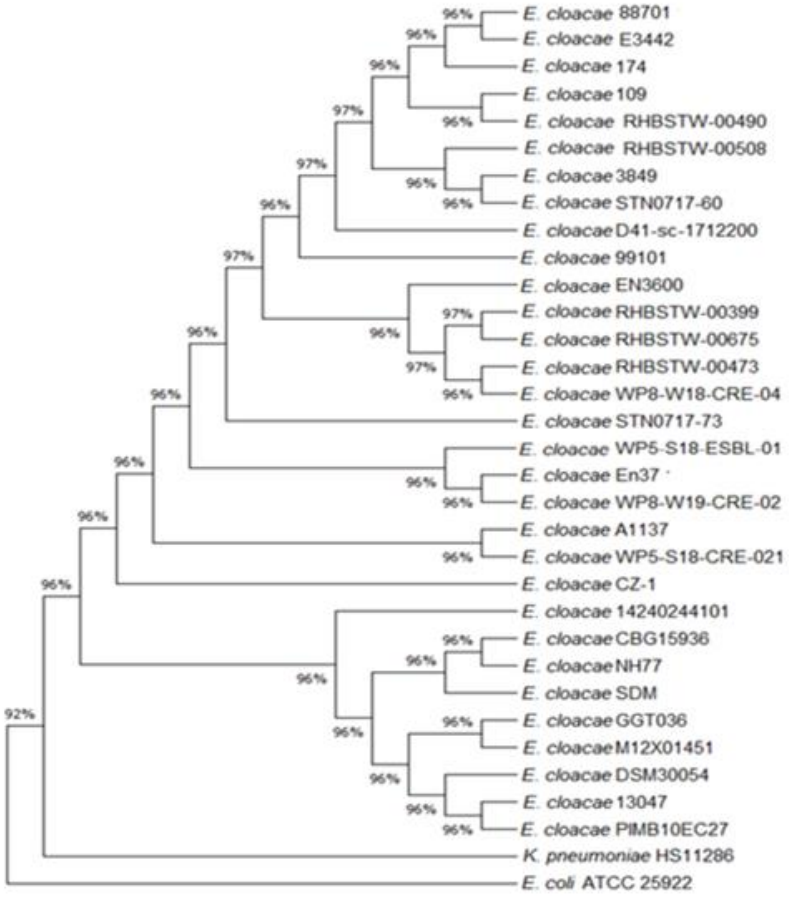
The genomes of the selected E. cloacae strains, in fasta format file, were submitted to the Comprehensive Antibiotic Resistance Database (Card), in which the BLAST method was used to perform the similarity search between the sequences that were submitted and those existing in the database (JIA et al., 2016). The parameters perfect and strict were selected, both related to the high percentage of identity between the sequences.

4 DEVELOPMENT

Phylogenetic tree

In the phylogenetic analysis, a high proximity rate was obtained between the strains analyzed, as shown in Figure 1. In the *bootstrap* values practically all strains presented 96% and 97% similarity, and only a single branch obtained the value of 92%. In the phylogenetic tree, the formation of five clades is observed, in the first one the formation of three *intra-clusters* can be identified (*E. cloacae* 88701 and *E. cloacae* E3442*)*, with isolates from a crustacean and bone infection, respectively. The second cluster was obtained from wastewater isolate (*E. cloacae* RHBSTW-00490) and tracheal aspirate (*E. cloacae* 109), and finally, the last cluster is formed by strains from hospital sewage (*E. cloacae* STN0717-60) and blood (*E. cloacae* 3849).

Figure 1: Phylogenetic tree with the 31 *Enterobacter cloacae* strains and the two reference strains (*K. pneumoniae* HS11286 and *E. coli* ATCC 25922).



The branches formed by strains of unavailable origin and bone infection (*E. cloacae* D41-sc-1712200 and *E. cloacae* 99101) are characteristic of non-monophyletic strains. The second clade is formed by two clusterizations and one isolated branch, in this clade most of the strains were obtained from wastewater from the UK (*E.cloacae* RHBSTW-00399, E.*cloacae* RHBSTW-00675 and *E.cloacae* RHBSTW-00473) and Japan (*E.cloacae* WP8-W18-CRE-04). The branch (*E.cloacae* EN3600) was obtained from a blood isolate. The third clade consists of a branch (*E.cloacae* WP5-S18-ESBL-01) with a strain from wastewater, and a *cluster* composed of a strain of unavailable origin (*E.cloacae* En37), and a wastewater isolate (*E.cloacae* WP8-W19-CRE-02).

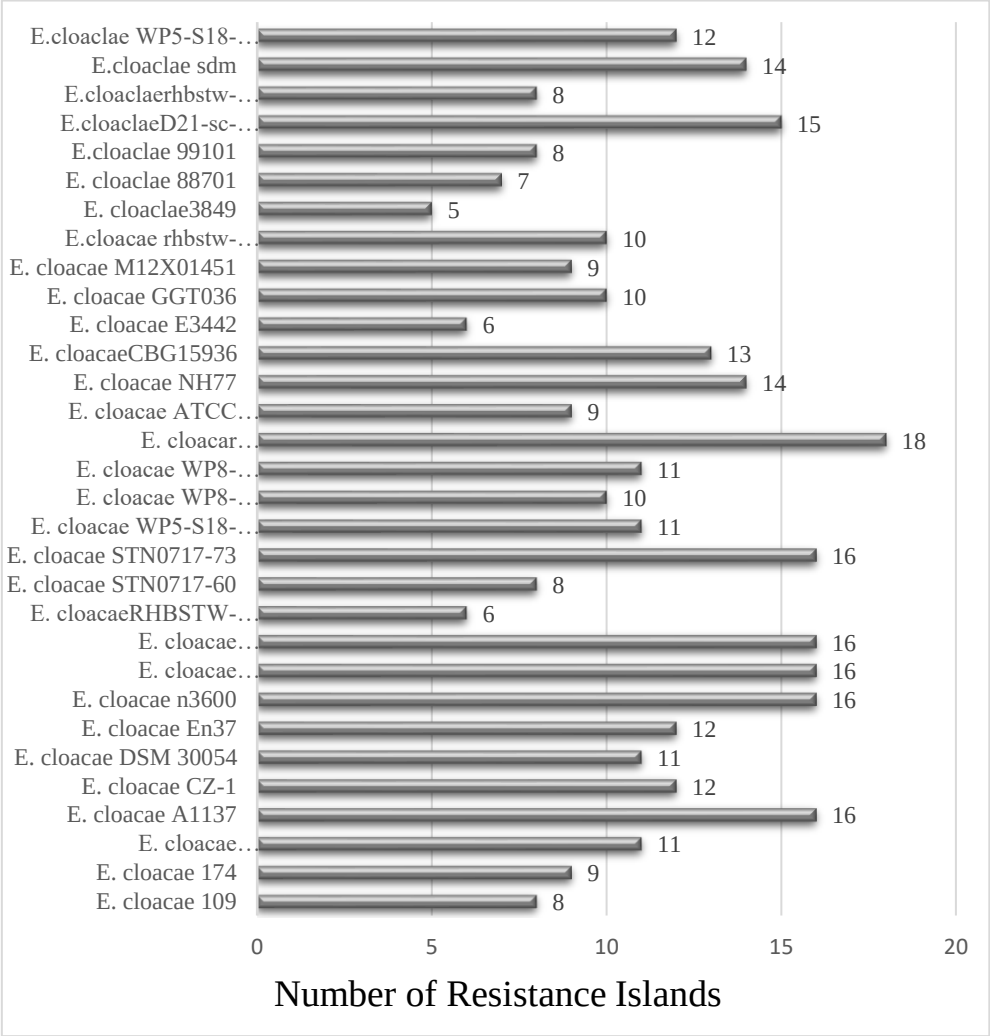
The fourth clade consists of a single *cluster* composed of strains isolated from blood and wastewater (A1137 and WP5-S18-CRE-021). Between some clades there are isolated branches, such as the branch formed by the strain from hospital sewage (*E.cloacae* STN0717-73) and the isolate from rice soil (*E.cloacae* CZ-1). Finally, the fifth clade is composed of an external branch obtained from bone infection sample (14240244101), and a *cluster*, presenting three *intra-clusters,* with the first cluster between a strain isolated from sputum and one of unavailable origin (*E.cloacae* CBG15936 and *E.cloacae* NH77), the second consisting of sputum and stool samples (E.*cloacae* GGT036 and *E.cloacae* M12X01451), and the third consisting of a spinal fluid isolate and a urine isolate (*E.cloacae*

ATCC 13047 and *E.cloacae* PIMB10EC27). The source of the other two branches (*E.cloacae* SDM and *E.cloacae* DSM 30054) present in the *cluster* is unknown.

Islands of resistance

Figure 2 shows the number of resistance islands that each strain has, and the genome of the 31 strains was analyzed in the Gipsy program with a focus on the identification of genomic islands of resistance. In this sense, it can be observed that the strain that presented the highest number of resistance islands has 18 islands in its genome (*E. cloacar* RHBSTW-00675) isolated in 2017 from a wastewater influent sample in the United Kingdom. Next are the strains *E. cloacae* EN3600, *E. cloacae* PIMB10EC27, *E. cloacae* RHBSTW-00399, and *E. cloacae* STN0717-73 with 16 resistance islands and with different origins.

Figure 2 - Number of Resistance Islands per strain. Source: Own authorship, 2021.



The *E. cloacae* STN0717-73 strain was found in Japan (2018) isolated from a hospital sewage tank sample (wastewater) and the *E. cloacae* RHBSTW-00399 strain was also isolated from aquatic material being wastewater in the UK in 2017. The other two strains have also been isolated from human biological material, where, *E. cloacar strain* EN3600 was found in China in 2015 by analyzing a blood sample (blood infection) and *E. cloacae strain* PIMB10EC27 originated from a urine sample (urinary tract infection) in 2010 at Binh Dan Hospital.

In addition, the strains with the lowest number of resistance islands (eight islands) were reported in human infections, among them, *E. cloacae strain* 109 isolated in the United States (2015) from a tracheal aspirate of a 42-year-old patient, and *E. cloaclae strain* 99101 isolated in the Netherlands (2017) through the collection of a bone infection. In addition, we can mention strains present in residual samples from the hospital environment such as the *E. cloacae* STN0717-60 strain isolated in 2018 in Japan from samples collected from a hospital sewage tank that also presents eight resistance islands and the *E. cloacae* RHBSTW-00490 strain discovered in the United Kingdom (2017) in freshwater samples near the wastewater treatment plant with six resistance islands in the genome.

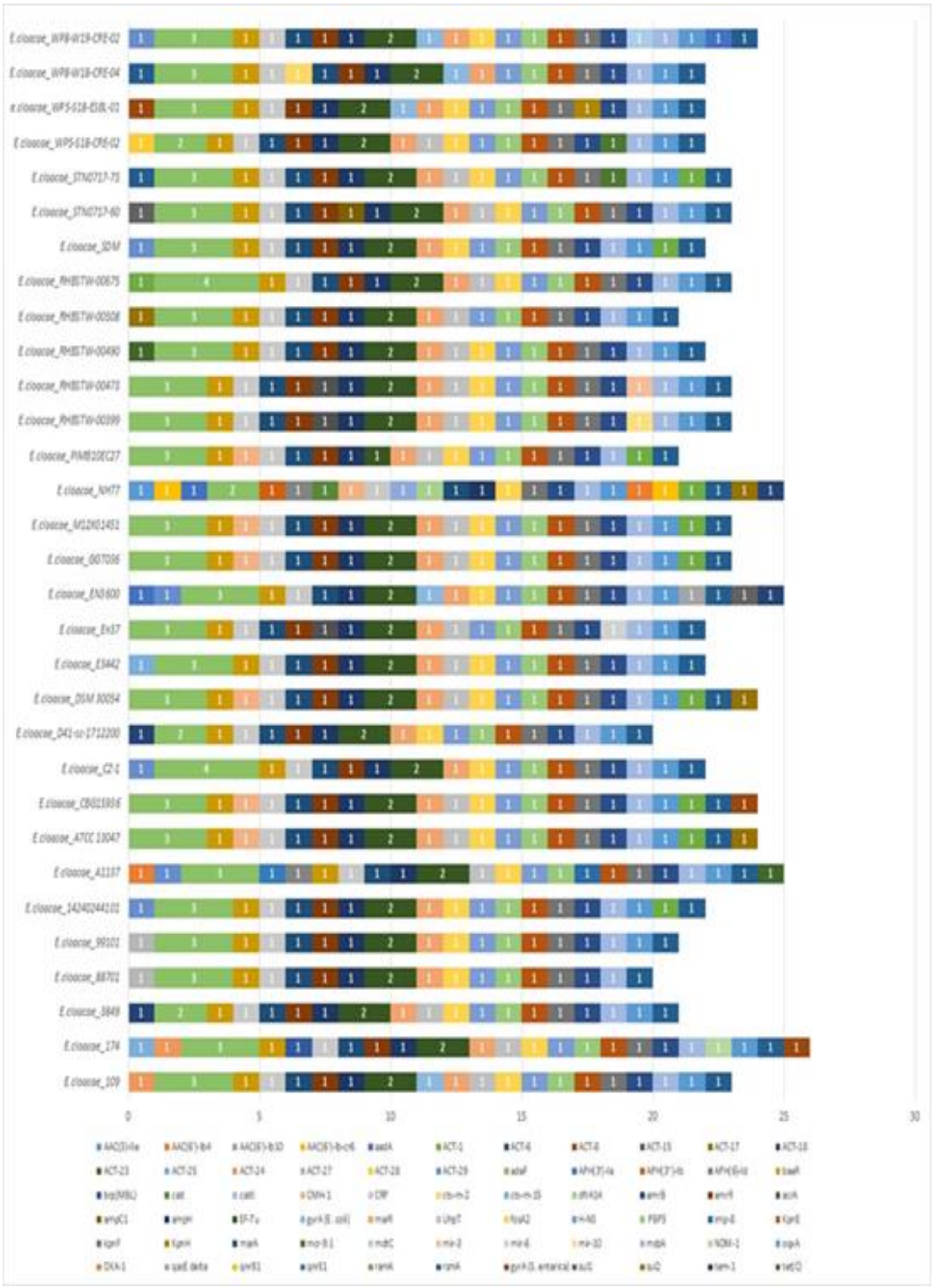
Analysis of antibiotic resistance genes

Regarding the resistance genes, the genes *adeF, baeR, crp, emrB, emrR, rsmA, msbA, marR, marA,* and *oqxA,* are present in more than 29 strains, and they are related to antimicrobial resistance through the antibiotic efflux mechanism, presenting resistance to the class of tetracyclines, nitrofuran and fluoroquinolone. Subsequently, it is observed that the *kpnE* and *kpnF* genes are present in more than 96% of the genome of the strains, are related to an increased susceptibility to cefepime, colistin and erythromycin, in addition, there is the *pbp3* gene that is found in 30 of the 31 strains and is associated with beta-lactamase resistance, that is, it is related to beta-lactam resistance through the mechanism of alteration of the antibiotic target.

The *ampH beta-lactamase* gene is present in 31 strains and the *fosA2 gene* is in 28 strains, both genes are associated with the process of resistance to beta-lactams and fosfomycin, respectively. Another gene that is present in 28 strains is the *oxqA gene* acting with the resistance mechanism through the antibiotic efflux pump. Also, it was analyzed that the *EF-Tur* gene (Thermo Unstable Elongation Factor) is found in 29 of the 31 strains, where it is characterized by producing proteins called Elongation Factors that act in the process of transporting aminoacylated tRNAs to the ribosome in the protein translation phase. It is also observed that about 93.5% of the strains have the *msdA* gene in their genetic material, where this gene presents resistance action due to the reduction of permeability to the antibiotic, among the antibiotics are phenicol, rifamycin, cephamycin, cephalosporin and carbapenem.

However, the figure shows that some genes are present only in specific strains as in the case of the *ampc1* gene that is in the *E. cloacae* STN0171-60 strain associated with antimicrobial resistance to class C beta-lactams.

Figure 3 - The strains are listed on the left side of the graph and the colors are used to differentiate the quantity and genes found in the microorganisms, at the bottom of the graph are the names of the genes with their respective colors. Source: Author, 2021.



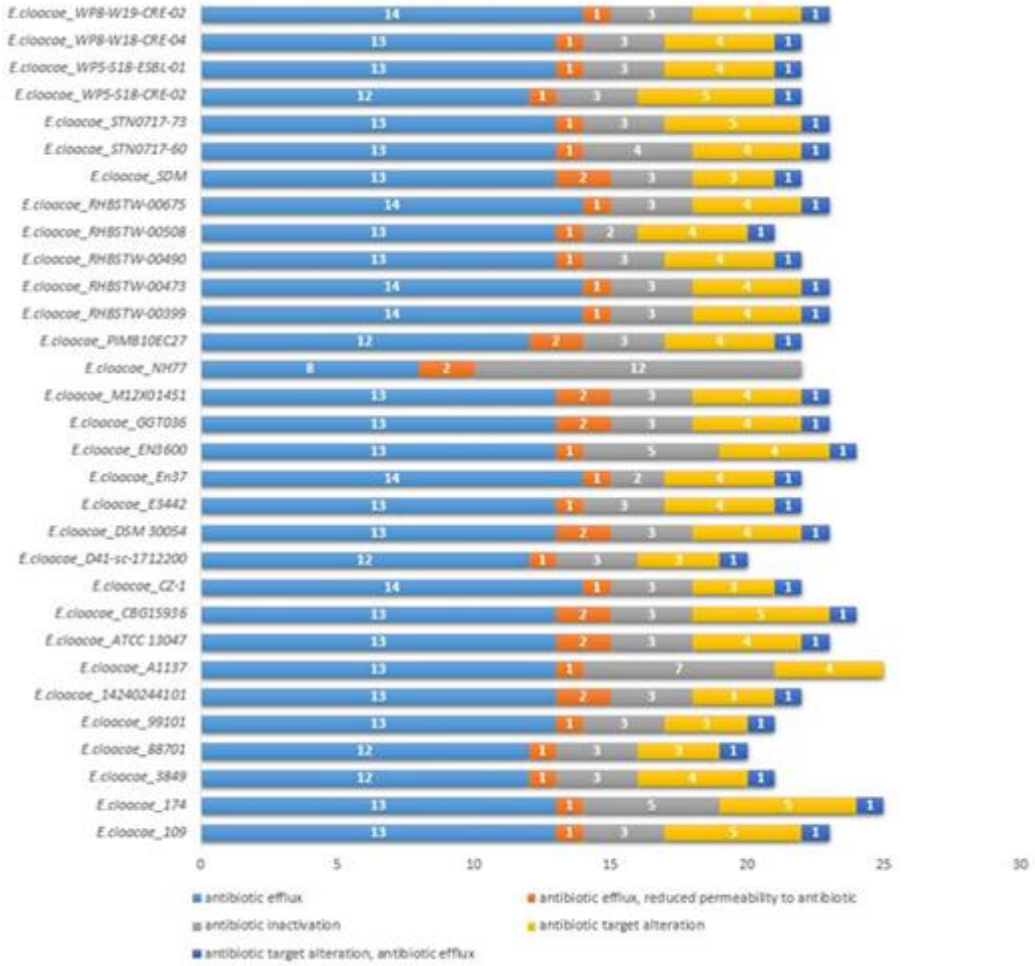
Aminoglycoside resistance occurs mainly from aminoglycoside-modifying enzymes, which are related to three groups: acetyltransferases (AAC), phosphotransferases (APH) and adenylyltransferases (AAD/ANT). In the present work, three acetyltransferase genes were found: *AAC(3)-IIe* and *AAC(6')-Ib-cr6* which are present in *E. cloacae* strain NH77; and the *AAC(6')-Ib 4* gene contained in the genome of *E.cloacae* A1137. In addition, it is noted that phosphotransferases are present in the same strains as acetyltransferases, thus, the *APH(3')-Ia* gene was found in E.*cloacae* A1137, while the APH*(3'')-Ib gene* is present only in *E. cloacae* NH77, finally, the *APH(6)-Id* gene is present in *E. cloacae* NH77 and *E.cloacae* A1137. Resistance to fluoroquinolones is triggered by a mutation allele, *aac (6 ') -Ib-cr,* which is being rapidly propagated due to an association with the *OXA-1* gene, which was found only in *E. cloacae* NH77.

The emergence of resistance to beta-lactams in the genus *Enterobacter* is associated with the emergence of enzymes such as beta-lactamases, a process associated with the genes bla_{TEM} and $bla_{\text{CTX-M}}$. In the present work, the *blaTEM-1* genes were found, present in the strains: *E. cloacae* NH77 and *E. cloacae* EN3600, and the $_{\text{blaCTX-M-2}}$ (*E. cloacae* WP8-W18-CRE-04) and $_{\text{blaCTX-M-15}}$ (*E. cloacae* NH77) genes. Another group of enzymes responsible for beta-lactam resistance are the metallo-beta-lactamases, associated with the genes bla_{NDM} *,* bla_{IMP} , among others. The genes $bla_{\text{IMP-8}}$ *and* $bla_{\text{NDM-1}}$ are present only in two strains of the study: *E.cloacae* A1137 and *E.cloacae* 174, respectively.

Analysis of resistance mechanisms

The resistance mechanisms of the study strains are represented in figure 4, in which it is observed that all strains exhibit the highest amount of resistance genes associated with the efflux pump, with the exception of *E. cloacae* NH77 which presented 12 genes related to antibiotic inactivation and 8 to the efflux pump. In addition, it is noted that all strains in the study have between 1 and 2 resistance genes associated with reduced permeability to the antibiotic. As for the inactivation genes, they were found in all *E. cloacae strains*, and in greater quantity in *E. cloacae* A1137 (7 genes) and *E. cloacae* NH77 (12 genes). Only one strain, *E. cloacae* NH77, does not contain a gene associated with the alteration of the antibiotic target, however the other strains present between 3 and 5 genes each. Regarding the genes related to the mechanism of target alteration together with the antimicrobial efflux pump, only *E. cloacae* A1137 and *E. cloacae* NH77 did not present any gene, while all the other strains presented a single gene for this mechanism.

Figure 4 - Mechanism of resistance presented by each strain. Source: Own authorship, 2021.



5 DISCUSSION

The phylogeny analysis of the strains showed that although some samples were isolated from different environments, such as hospital and environmental environments, the strains are grouped in the same cluster. Thus, strains from different environments and even different geographic locations have genetic similarities, however, it is noteworthy that this analysis was performed with the 16S ribosomal gene. This gene is used in phylogenetic analyses to identify strains belonging to the same species, being efficient to infer the phylogeny of bacteria, due to the high conservation of the sequence, allowing the visualization of the relationships developed by microorganisms (Talal, Hiba, Mohammed, 2021). Thus, this result is expected since this gene undergoes few variations among the various strains.

It is worth mentioning that the strains presented a high similarity, ranging between 96% and 97%, according to Straliotto (2006), proximity values of 70% homology indicate at least 96% similarity between the sequences. Similar analyses had been previously performed, as in the work performed by Liu et al., (2013), who used the 16S ribosomal gene in the comparative analysis of strains isolated from different environments, whether hospital or environmental, and obtained a high similarity (>99%). The study conducted by Mshana et al. (2011), about a new strain of *Enterobacter* spp., from the 16S gene, also obtained high proximity between the strains studied (>90%).

Islands

Bacteria can present regions called genomic islands, where they are regions of exogenous DNA acquired by horizontal transfer, such regions are classified according to the gene composition, so there are islands of metabolism, genes related to the symbiotic action of bacteria to other organisms, islands of pathogenicity and islands of resistance, In this sense, in relation to islands of resistance the literature divides into three main mechanisms the process of acquisition by bacteria, they are by horizontal, vertical transfer and by spontaneous mutation (Soares, et al., 2015; Santos, 2019;).

In this context, the results showed two strains originating from human bone infections and presenting eight resistance islands (*E. cloaclae_99101; E. cloacae_109*). It is interesting to report that Health Care-Related Infections (HAI) have drawn the attention of public health agencies, as the hospital environment has been presented as a reservoir of bacteria and makes the environment susceptible to the transmission of pathological agents to immunosuppressed patients even after the sanitization of hospital materials and inanimate surfaces that may present bacteria multidrug-resistant to antibiotics. Transmission can occur due to the presence of microorganisms left by patients previously hospitalized in the same bed, relatives, and also by health professionals who pass through the place (Da Silva, Xavier, Roder, 2020).

Thus, the elucidated result is pertinent, since studies such as the one developed by Chaoui et al. (2019) who collected 241 samples with a swab pre-moistened in sterile normal saline water in 148 public hospital beds on operating tables, beds, medical devices, walls, floors and sinks resulted in the identification of several pathogens, including Gram-negative bacteria such as *Enterobacter* spp. and Acinetobacter spp, *Klebsiella* spp. and *Acinetobacter spp.* Another study carried out by Alamari and collaborators (2020) in Intensive Care Units reported the transmission of pathogens in the hospital environment through patients, the hospital environment and the hands of health professionals, and more, that humid and low temperature places contribute to the long duration of microorganisms on the surface, in addition to the great use of antibiotics in the treatment of patients. patients. Therefore, the result presented on the origin of the strains and amount of resistance islands, is expected, due to all the circumstances that the environment provides for contamination, transmission and development of resistance in the hospital environment.

An important result presented in the analysis of resistance islands presented by strains originating from aquatic environments is highlighted, as in the case of the *E. cloacae RHBSTW-00675 strain,* which presented in its genetic material the largest amount of resistance islands along with other highly representative strains. In addition, in a study published by Zhang et al. (2015) the authors state that aquatic environments demonstrate greater ease of exchange of genetic material between pathogenic and environmental bacteria, giving rise to strains that are multidrug resistant to various antibiotics. It is interesting to comment on how human action can influence the development of

antibiotic resistance, considering that the aquatic environment can be contaminated by different classes of antimicrobials, as reported in the study by Kolpin et al. (2002) that at the end of the work approximately five classes of antibiotics were identified in surface water samples, among them are macrolides, fluoroquinolones and tetracylins.

Thus, it is relevant to increase care with the treatment of hospital sewage, since the present work elucidated the identification of strains such as *E. cloacae* STN0717-73 (found for the first time in the hospital sewage tank) with 16 islands of resistance. In addition, it is known that the hospital environment has several pharmacological treatments with antimicrobials and needs correct disposal, and also, an effective treatment so that the nearby water regions (lakes, rivers and streams) are not contaminated, because, the aquatic environment presents itself as the ideal place for the spread of antibiotic resistance, proving to be an important region for sharing antibiotic resistance genes in natural ecosystems, resulting in the contamination of water sources and even soils near the source (Gillings, 2008; Alves, 2019).

In the analysis of resistance mechanisms, the most frequent among the strains was the efflux pump, discovered in the 1980s. This mechanism was initially associated with resistance to tetracycline, however it is now known to be correlated with resistance to virtually all antibiotics, and can be found in both Gram-positive and Gram-negative bacteria, being stronger in the second group. Some efflux pumps selectively export specific antibiotics, while others, called MDR (Multiple Drug Resistance), already expel diverse structures, and at high concentrations, causing the drug not to remain at optimal levels for effective functioning. Thus, this mechanism is responsible for both intrinsic and acquired resistance to several antibiotics (Lomovskaya, Bostian, 2006; Santajit, Indrawattana, 2016).

Efflux pumps had already been pointed out as the most frequent mechanism in other studies, such as the one carried out between 1995 and 2003, in which a significant increase in this mechanism was noted in the period in question, in *Enterobacter aerogenes* (Chevalier et al., 2008). As well as in the study carried out by Olga Lomovskaya and collaborators (1999), in which it was observed that the frequency of resistance triggered by efflux pump was higher than that caused by target alteration in Gram-negative bacteria, such as *Pseudomonas aeruginosa*. Furthermore, one study highlighted the increased prevalence of efflux pumps as resistance mechanisms in the species; *Enterobacter aerogenes* and *Klebsiella pneumoniae* (Santajit; Indrawattana, 2016). Another work, this one carried out with strains of the *E. cloacae* complex, identified that efflux pumps were the resistance mechanism most associated with resistance to carbapenems (Liu et al., 2021).

Genes

Among the most identified genes in the present study, identified in 29 strains, was *oqxA*, responsible for encoding a multidrug efflux pump of the RND family, OqxAB, recently identified in

E. cloacae and responsible for the decline in susceptibility to Quinolones in *Enterobacteriaceae* (Hansen, et al., 2005; Davin-Regli,; Lavigne, Pagès, 2019). Therefore, the resistance genes *baeR, crp, emrB, emrR, msbA, marR, marA and PBP3* associated with antibiotic efflux pump coding were detected in approximately 94% of the strains, being responsible for resistance to treatments using aminoglycosides (*baeR*), penicillin (*crp, marA, PBP3, marR*), cephalosporins (*marA, marRm PBP3*), monobactams and carbapenems (*marA* and *PBP3*), macrolides (*crp)* and fluoroquinolones (*crp, emrB, emrR, marA, marR*) (Xi Yap, et al., 2020). In this context, it is analyzed that a large part of the strains found of *E. cloacae* demonstrate resistance to a wide diversity of antimicrobials, showing itself as a species resistant to conventional treatments.

Another mechanism of resistance present in the strains of the study was reported, which is the inactivation of antibiotics, which, although frequently reduced in relation to the efflux pump, is highlighted in view of the fact that 28 of the 31 strains presented the *fosA2 gene, a* gene that acts in the inactivation process by breaking the epoxide ring of the fosfomycin molecule. In a study by Chen et al. (2021) it is reported that the *fosA2* gene has lower fosfomycin inactivation activity than *fosA3* and a stronger potential for propagation. However, *fosA2* has among its genetic origins transmission by plasmids and horizontal transmission. Thus, it is interesting to comment that in a work done by Harada and collaborators (2018) isolates of *E. cloacae* were found in ready-to-eat foods and vegetables that also presented in their genetic structure the *fosA gene, in* this sense, it shows how the strain presents diverse origins and the presence of an inactivating gene of an important antimicrobial in the treatment of infections.

ESBLs are responsible for resistance to extended-spectrum cephalosporins (ESCS) and monobactams, and the main enzymes of this group are derived from the genes, bla_{SHV} , bla_{TEM} and $bla_{CTX\text{-}M}$ (Souna et al., 2014; Annavajhala, Gomez-Simmonds, Uhlemann, 2019). Thus, the genes $_{blaCTX\text{-}M\text{-}2}$, $bla_{TEM\text{-}1}$ and $_{blaCTX\text{-}M\text{-}15}$, deserve to be highlighted, despite being found in few strains in this study. (2014), with ESBL-producing *E. cloacae* isolates, it was identified that most of the strains in the study had $bla_{CTX\text{-}M}$, but specifically $_{blaCTX\text{-}M\text{-}15,}$ while no strain had bla_{TEM} . A second study, this one conducted in Iran, with *E. cloacae* isolates, again $bla_{CTX\text{-}M\text{-}15}$ was the most commonly found gene, followed by the $_{blaTEM\text{-}1}$ gene (Peymani et al., 2014).

Metallo-beta-lactamases (MBL) are enzymes related to resistance to carbapenems, including meropenem, ertapenem and imipenem, with the latter being considered the most effective antibiotic in the treatment of *E. cloacae* infections, and the genes most related to the production of these enzymes are bla_{VIM} , bla_{IPM} and bla_{NDM} (Cai et al., 2019; Davin-Regli, Lavigne, Pagès, 2019). Of the genes mentioned, only bla_{IPM} and bla_{NDM} were found in two strains of the study, despite the small number, the importance of drugs for *E. cloacae is* highlighted. The work developed by Cai and collaborators (2019), identified the presence of bla_{IPM} and bla_{NDM} in *E. cloacae* isolate, but specifically the $bla_{NDM\text{-}}$

1 gene that was the most prevalent in the study, and variants of *bla*$_{IPM}$. A second work, this one conducted in China, found five strains containing *bla*$_{IPM-8}$ and a single strain containing *bla*$_{NDM-1}$, among *E. cloacae* isolates obtained from a hospital (Dai et al., 2013).

6 FINAL CONSIDERATIONS

Based on the above, it can be seen that in the phylogenetic analysis a similarity above 90% was obtained among the strains studied, which corroborates with data previously exposed. In this sense, the analysis of the resistance islands of the 31 strains showed that the strains that exhibited the highest number of resistance islands come from aquatic samples with about 16 to 18 resistance islands. In this context, it is important to emphasize the origin of the isolates, as in the case of *E. cloacae_STN0717-73, a* strain isolated from hospital sewage with 16 islands of resistance in its genetic material, with this, it is relevant to direct attention to effective sanitary treatments in view of the risk presented by hospital sewage, to prevent contamination of water sources and regions close to hospitals.

In addition, the main resistance mechanism corresponds to efflux pumps, found in all 31 strains, and similar data have already been found in other studies with the same species, and with other species within the genus *Enterobacter*. Regarding the resistance genes identified in the strains, nine genes can be highlighted (*crp, emrR, emrB, msbA, marR, marA* and *PBP3*) shared by about 29 to 31 strains and which are related to the resistance mechanism through the efflux pump presenting resistance to cephalosporin, macrolides, fluoroquinolones and carbapenems.

In addition, the *fosA* (28 strains) and *ampH* (31 strains) genes that act through the inactivation of antibiotics are identified. Less frequent genes were found in the study, but they are of clinical importance, as they are associated with resistance to beta-lactams and carbapenems, especially the *bla* gene$_{IPM-8}$, indicative of the emergence of resistance to the antimicrobial Imipenem, a drug of last resort, and one of the most effective in the therapy of *Enterobacter cloacae* infections.

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