

Phenotypic and genotypic profile of Enterobacteria resistant to beta-lactams

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Abstract

Background: A serious emerging problem worldwide is increased antimicrobial resistance. Acquisition of coding genes for evasion methods of antimicrobial drug mechanisms characterizes acquired resistance. This phenomenon has been observed in several bacteria, including major species of Gram-negative bacteria such as the Enterobacteriaceae family. Among these, strains resistant to multiple classes of antibiotics were observed. Treatment for bacterial infections is performed with antibiotics and among the most used beta-lactams stand out. Resistance to this class of antimicrobials has also increased. The aim of this study was to correlate antimicrobial resistance profiles in Enterobacteriaceae by phenotypic methods and molecular identification of 14 beta-lactamase coding genes: blaOXA; blaIMP; blaNDM; blaSME; blaDHA; blaCMY, blaTEM, blaKPC, blaSPM, blaCTX-M, blaVIM, blaSIM, blaGIM, and blaSHV.

Methods: The phenotypic methodologies used were the Antimicrobial Sensitivity Test for Disk-Diffusion, and complementary tests for the detection of resistance mechanisms of beta-lactamases (ESBL, MBL, AmpC and Carbapenemase). The molecular methodology used was Real Time PCR using the Sybr Green system.

Results: Among the results found in the tests it was observed that 74.28% were resistant to ampicillin, 34.28% were resistant to aztreonam, 62.85% were resistant to amoxicillin associated with clavulanic acid, 51.42% were resistant to ceftazidime, 41.42% were resistant to ceftiofur, 54.28% were resistant to cefazolin, 44.28% were resistant to cefepime, 41.42% were resistant to cefuroxime, 8.57% were resistant to cefuroxime, 35.71% were resistant to imipenem and 41.42% were resistant to piperacillin associated with tazobactam. Among the total samples, the mechanism of resistance that presented the highest expression was ESBL (17.14%). The genes studied that were detected in a greater number of species were blaGIM and blaSIM (66.66% of the samples). The gene that was amplified in a smaller number of samples was blaVIM (16.66%).

Conclusions: It is concluded that although there is a low correlation between the methodologies analyzed, the levels of antimicrobial resistance in enterobacteria are high and worrying, and a way to minimize the accelerated emergence of resistance includes the development or improvement of techniques that generate diagnoses with high efficiency and speed.

Background

Currently, in relation to taxonomy, the Enterobacteriaceae family has 53 genera of which more than 170 species have already been named. Among these, 26 bacterial genera have already been associated with bacterial infections in humans. Members of this family are small gram-negative, facultative anaerobic rods and most species are able to grow at 37 °C, although some grow more properly at 25 to 30 °C (1).

These microorganisms are widely distributed in nature and are found in soil, water, vegetables, in the intestinal tract of humans and vertebrates (2). Enterobacteriaceae represent the main group of bacteria isolated in clinical samples and are associated with a wide variety of community and hospital infections (3). Gram-negative bacteria, specifically Enterobacteriaceae, are common causes of both community-acquired and hospital acquired infections, including urinary tract, bloodstream, and lower respiratory tract infections (4).

Resistance among clinically important organisms to antimicrobial agents is severely threatening the repertoire of treatment options for common infections. The challenge is intensified by the fact that several of these organisms are resistant to multiple antimicrobials (5). Infections caused by Gram-negative bacteria resistant to multiple drugs are a serious public health problem due to the scarcity of treatment options for these infections (6).

Antibiotics play a key role in the success of some medical practices. Unfortunately they tend to lose their efficacy over time due to the emergence and spread of resistance among bacterial pathogens (7).

Drug resistance genes can be spread from one bacterium to another through various mechanisms such as plasmids, bacteriophages, naked DNA or transposons. Some transposons contain integrons—more complex transposons that contain a site for integrating different antibiotic resistance genes and other gene cassettes in tandem for expression from a single promoter (8). Bacterial conjugation is the most sophisticated form of horizontal gene transfer (HGT) in bacteria and provides a platform for the spread and persistence of antibiotic resistance and virulence genes (9).

Beta-lactams are preferred because of their clinical efficacy and safety by virtue of their high selective toxicity (10). Resistance to beta-lactams in Enterobacteriaceae and other Gram-negative organisms is primarily mediated by beta-lactamases (11). Beta-lactamases are enzymes that catalyze the hydrolysis of the beta-lactam ring inactivating the antimicrobial and preventing it from being active against the enzymes responsible for bacterial cell wall synthesis (12).

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system (13). The antibiogram provides qualitative results by categorizing bacteria as susceptible, resistant intermediate or resistant. Therefore, it is a tool based on the resistance phenotype of the tested microbial strain. However, inhibition of bacterial growth does not mean bacterial killing, the phenotypic method fails to distinguish between bactericidal and / or bacteriostatic effects (14).

Molecular diagnosis is another method of identifying bacterial resistance that can be applied. The molecular technique performed through nucleic acids, while requiring advancements, may allow a patient to obtain the result of an examination rapidly, within a four-hour period; thus, initiating the most appropriate antibiotic therapy. This can improve treatment outcomes for the patient and reduce empirical antimicrobial prescriptions, decreasing the duration and cost of antimicrobial treatment. Thus, technologies with the diagnosis of nucleic acids have the potential to reduce the selection of new resistances as well as to reduce the potential of existing resistances (15).

The objectives of this study are to correlate the resistance profiles of Enterobacteria using phenotypic and genotypic methodologies. The genes encoding resistance to beta-lactams are: *bla*SPM, *bla*SIM, *bla*VIM, *bla*KPC, *bla*SHV, *bla*CTX-M, *bla*GIM, *bla*OXA, *bla*IMP, *bla*NDM, *bla*SME, *bla*DHA, *bla*CMY and *bla*TEM. This study is justified because it is assumed that molecular methods improve accuracy, efficiency and accuracy compared to the classical phenotyping method. In addition, it can be released in a short time; helping to improve the effectiveness of antibiotic therapy.

Methods

A total of 70 bacterial samples of Enterobacteriaceae were stored in a bio-repository at the Laboratory. Among the analyzed bacteria are the species: *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter freundii*, *Morganella morgani*, *Providencia* spp., *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Raoultella terrigenes*, *Escherichia coli*, *Escherichia blattae*, *Edwardsiella ictaluri*, *Cedecea neteri*, *Erwinia persicina*, *Providencia rustigiani*, *Salmonella paratyphia*, *Salmonella typhi*, *Yersinia ruckeri*, *Serratia marcescens* and *Hafnia alvei*. Bacteria come from mucosa of human tonsils (five samples), human corneas (twenty three samples), animal bladder (four samples), animal uterus (ten samples), Veterinary Hospital Environment (twenty one samples) as well as respiratory equipment from a hospital service, Manual Resuscitators - MRI (seven samples). The isolates of human tonsils originated from Hospital of the clinics of the Federal University of Goiás, Brazil; human corneas from the Service of Verification of Deaths (SVO) of Goiânia, Goiás, Brazil; the animal bladder and uterus samples were obtained from a female dog hospitalized veterinary hospital of Goiânia, Goiás, Brazil; veterinary hospital environment samples came from the Dog Center clinic in Goiânia, Goiás, Brazil and manual resuscitators from an Intermediate Care Unit (ICU) of a public hospital in the state of Tocantins, Brazil.

After being stored as a bio-repository, these enterobacteria were randomly used in this study to compare the resistance profile presented by both phenotypic and genotypic methodology.

The antibiogram and sensitivity of the Gram-negative bacilli samples to the various antimicrobials were performed according to agar-diffusion methodology (Kirby-Bauer), according to the bacterial genus were used the antimicrobials ampicillin 30 µg, amoxicillin-clavulanate 20/10 µg, aztreonam 30 µg, cefazolin 30 µg, cefepime 30 µg, ceftazidime 30 µg, ceftriaxone 30 µg, imipenem 10 µg and piperacillin-tazobactam 100/10 µg. As a quality control, strains *E. coli* ATCC® 35218 were used for combinations of β-lactam inhibitors / β-lactamases (16).

For the phenotypic detection of Extended spectrum beta-lactamases (ESBL) production, the enterobacteria was isolated and submitted to the approach disk technique, modified using the methodology described by Jarlier et al. (17).

For the phenotypic detection of AmpC-type beta-lactamase, the induction test was performed using antimicrobial susceptibility testing, was performed by the disk diffusion assay (Kirby–Bauer technique) according to the 2015 European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations (18).

Imipenem and meropenem discs were used for the phenotypic investigation of carbapenemases and the interpretation of the sensitivity following the criteria established by CLSI (16). At least one bacteria that showed resistance of the carbapenems were submitted to the MBL screening test, using the enzyme blockade method and following the recommendations of ANVISA (18). The test used imipenem (10µg) and meropenem (10µg) disc, positioned parallel to two other imipenem and meropenem discs added with 10µL of EDTA.

For enterobacteria, in addition to the EDTA test, the modified Hodge test (MHT) was also performed. MHT consists of the inoculation of *E. coli* ATCC 25922® on the entire surface of a Müller-Hinton agar plate. A meropenem disk was placed in the center of the plate and around this disk streaks were made with the suspected samples, as recommended by CLSI (16).

For each bacterium, plasmid extraction was done according to the FLEXIPREP extraction kit manual from Pharmacia®, according to the manufacturer's instructions. For the qPCR assays, specific primers were designed based on the sequences deposited in GenBank (Table 1).

Reactions were prepared using the Sybr Green (Sybr Green qPCR master mix LOWROX - 100 reactions x 25 uL) Real Time PCR kit, following methodology suggested by the manufacturer. For the positive and endogenous control of the reaction the primers were used to amplify the 16S RNA, for the negative control, water was added in place of the DNA. Fisher's test was used to compare the techniques considering isolated samples.

Purified plasmid DNA preparations were digested with restriction enzymes for identification and characterization of the genes of that study according to the preparation: in microcentrifuge tubes were added: 2 µl of 10x Buffer (Ludwigbiotec), (Buffer EcoR I for enzyme EcoR I and Buffer V2 for Hind III); 1 µl of EcoR I or Hind III enzyme (10 UI / µL) (Ludwigbiotec), 15 µL H₂O; 2 µl template DNA (~300 ng / µl). The tubes were placed in thermoblocks at 37 °C overnight and were then incubated at -20 °C for 15 minutes. From these preparations agarose gel electrophoresis was performed, as controls were used the preparation without the enzyme and a non-incubated preparation.

Results

The enterobacteria from this study were isolated from samples collected in four different types of origin. Among the total bacterial isolates, 40% are from human clinic, 20% from animal clinic, 10% from human hospital environment and 30% from veterinary hospital environment.

This study of antimicrobial resistance in enterobacteria characterized a phenotypic profile of resistance to beta-lactam antibiotics, in which among the 70 bacterial samples studied, 52 (74.28%) were resistant to ampicillin, 44 (62.85%) were resistant to amoxicillin associated with the beta-lactamase inhibitor clavulanic acid, 38 (54.28%) were resistant to cefazolin, and 6 (8.57%) were resistant to cefuroxime. The Table 2 shows the percentage of antimicrobial resistance by sample source of Enterobacteriaceae.

Phenotypically, through the antibiogram method, the species *E. aerogenes*, *E. agglomerans* and *C. neteri* stand out exhibiting the highest rate of resistance, being resistant to 10 of the 11 antibiotics tested (90.9%).

The MDR, XDR and PDR profiles, found phenotypically in this study, determined that among the enterobacteria studied here there was a predominance of 68.57% XDR profiles, in which there was sensitivity to at least two groups among the four that form the β-lactams. These data can be observed in Figure 1.

Fortunately no PDR profile was found because no bacteria were resistant to all antibiotics but because the acquisition of antimicrobial resistance is a natural phenomenon and can be accentuated by other factors, it should not be ruled out that they may present new profiles in the future including the PDR profile.

Still on the phenotypic profiles, it was observed that 28.57% were MDR and that the penicillin group was the antibiotic for which there was the highest resistance rate. There was resistance to at least one of the penicillins studied here in approximately 85.71% of the bacteria. For cephalosporins, there was resistance to at least one of those tested here in 77.14% of bacteria, a relatively high number demonstrating that such drugs, from the first to the fourth generation, are also losing their effect on enterobacteria.

In relation to β-lactams used as drugs of last resource- carbapenems - in this study there was phenotypic resistance to imipenem in 35.71% of the isolated bacteria. The antibiotic that presented the lowest percentage of resistance was the monobactam aztreonam with a resistance rate of 34.28%, which corresponds to a rate close to that of carbapenems, showing that these antibiotics were the most effective against most bacterial samples studied.

The specie *E. agglomerans* showed phenotypic resistance data with profiles ranging from completely sensitive to XDR. This shows that within a same bacterial specie the resistance possibilities are very variable.

Following the analyzes of the results of this work, the mechanism of greatest strength that has been the ESBL (17.14%), while the others were: AmpC (4.2%), MBL (1.4%) and Carbapenemase (0%). The specie *Y. ruckeri*, was the one that promoted more number of mechanisms (ESBL, MBL and AmpC). The species that did not present any of the mechanisms were: *E. blattae*, *H. alvei*, *R. terrigena* and *C. freundii*.

The percentages of amplification of the beta-lactamase genes, through the qPCR method, found among Enterobacteriaceae were: 66.66% for the *blaGIM* and *blaSIM* genes, 61.11% for the *blaDHA* and *blaTEM* genes, 55.55% for the *blaCMY*, *blaCTX-M*, *blaNDM*, *blaOXA* genes,

50% for the *bla*IMP gene, 44.44% for the *bla*SHV and *bla*SPM genes, 38.88% for the *bla*KPC gene, 33.33% for the *bla*SME gene and 16.66% for the *bla*VIM gene.

The phenotypic profile of resistance to beta-lactam antimicrobials was determined in 19 bacterial species among enterobactérias: among all the phenotypic resistances found, the species *E. aerogenes*, *E. agglomerans*, *C. freundii* and *C. neteri*, stood out showing the highest resistance rate (90.9%). The molecular profile of resistance to beta-lactam antimicrobials was determined in 18 bacterial species among enterobacteria: 94.44% showing resistance for aztreonam, ceftazidima, ceftoxitin and piperacillin associated with beta-lactamase inhibitor tazobactam. Phenotypic and molecular data are compared in Table 4.

By analyzing the amplification rate of the genes that confer beta-lactam resistance and making an association of the same with the literature review carried out in this study, it was observed that the species that showed potential for resistance of a greater number of antibiotics were: *E. aerogenes*, *R. terrigena*, *M. morgani*, *E. ictaluri*, *C. neteri*, *S. paratyphi* and *Y. ruckeri*., exhibiting resistance potential for all antibiotics (100%) tested. *C. freundii*, *Klebsiella* spp., *E. coli*, *E. blattae*, *P. rustigiani* and *E. persicina* were studied, showing resistance potential for 10 of the 11 antibiotics (90.9%) tested.

The resistance information obtained in this study shows that 100% of the analyzed species present a high potential for resistance to several beta-lactams. Among the potential profiles suggested by the qPCR analyzes, the XDR and PDR data were, respectively, 67,14% and 32,8% among the studied species. These data can be observed in Figure 1.

In molecular analyzes, PDR should be considered. The rate found for these pages was 38.88% and, although not very high, has repercussion on the degree to a clinical problem that may represent for the population.

The Pearson coefficient was calculated to linearly correlate two variables. The Pearson correlation coefficient varies between -1 and 1. The signal indicates the direction of correlation (negative or positive) while the value indicates the magnitude. The closer to 1 the stronger the level of linear association between variables 3.

In this study the detection rate of antimicrobial resistance by molecular methodology was generally higher than the detection rate by phenotypic methodology. But as already mentioned, the mere presence of the resistance gene in the bacterial genome does not necessarily imply its expression, and the phenotypic methodology is still necessary.

Were done a experiment to verify the plasmid perfil, and from plasmid DNA digested were no identified sites to *Eco*R I and *Hind* III restriction enzymes. After these results the authors decided that the best experiment to observe the restriction plasmid profile must be the sequencing experiments that will be done in another study.

Discussion

The phenotypical results of the present study are in agreement with a retrospective study that was carried out in a laboratory of clinical analyzes of Goiânia, Goiás, which evaluated the prevalence and antimicrobial susceptibility profile of the isolated microorganisms from 432 samples, in which the species *E. aerogenes*, *E. agglomerans* and *C. neteri* are related among those that present resistance to multiple drugs (20).

With the exception of *C. neteri*, which is a strain of animal origin, these bacterial species are in accordance with epidemiological data indicated by ANVISA (19), as they are among the species of enterobacteria most prevalent in primary bloodstream infections associated with the use of catheters in hospitalized patients in adult, pediatric and neonatal ICUs in Brazil.

About multidrug resistance (MDR), extremedrug resistance (XDR) and pandrug resistance (PDR) profiles have recently been updated by the CDC (Center for Disease and Control) and ECDC (European Center for Diasses Control and Prevention), promoted with the objective of international standardization of these terminologies, as published by Magiorakus et al., (20), in which, MDR was defined as the resistance to at least one agent in three or more categories of antimicrobials, XDR is resistance to at least one agent, including all categories, and PDR is resistance to all agents in all categories of antimicrobials.

According with authors, Enterobacteria resistant to carbapenems typically have XDR phenotypes and infections are associated with high mortality rates (up to 70%), making them particularly challenging from a clinical standpoint (7).

A study corroborates with our data: it was analyzed the epidemiology of IPCSL and the sensitivity profile of the microorganisms of the State of Goiás in 2016, percentages on resistance were: 75% *Enterobacter* spp. resistant to cephalosporins of 4th generation, 50% of *E. coli*

resistant to carbapenems and to cephalosporins of 3rd and 4th generation; 28.6% of carbapenem resistant *K pneumoniae* and 3rd and 4th generation cephalosporins and 42.9% resistant to 3rd and 4th generation cephalosporins, in addition to 50% of *Serratia* spp. resistant to the 3rd and 4th generation cephalosporins; differing from national results (23). Carbapenem-resistant enterobacteria have emerged as a major cause of nosocomial infections worldwide and are characterized by rapid and progressive dissemination (24).

In a study of carbapenem-resistant enterobacterial isolates (CRE) isolated from patients who received medical care at Stanford Health Care and Lucille Packard Children's Health, California, USA, between January 2013 and December 2016, as carbapenem minimum inhibitory concentration (MICs) for the CRE card ranged from ≤ 1 to $265 > 8 \mu\text{g} / \text{mL}$ for imipenem, which also demonstrated increased resistance to this antimicrobial (25).

A study carried out in northeastern Brazil showed that in 672 urocultures positive for urinary tract infection, the etiological agent belonged to the Enterobacteriaceae family in 86.9%, and among them 29 (4.8%) were ESBL (26).

A literature review was carried out to determine the potential of resistance by molecular methodology found in this study, which has genes described in the literature that encode the β -lactamase enzyme. The result of the literary survey is shown in Table 3 (27–93).

In a study carried out with clinical isolates of carbapenem-resistant Enterobacteriaceae collected at the University Hospital of Santa Maria, Rio Grande do Sul, Brazil, the *blaKPC*, *blaOXA-48*, *blaNDM*, *blaSPM*, *blaIMP*, *blaVIM* and *blaGIM* genes were investigated by PCR and multiplex PCR. About the number of studied microorganisms, the genotypic tests evidenced that *blaKPC* was the most encountered gene, in 31% (n = 10) of the samples, followed by *blaIMP*, in 12.5% (n = 4) (94).

In a study conducted in eight hospitals in the Paris region of France, twelve isolates were collected in twelve patients, 11 *K. pneumoamiae* and 1 *K. oxytoca*. All isolates showed *blaDHA* gene and (4/12) 33.33% *blaTEM* gene (95).

In another study, a total of 88 phenotypically ESBLs positive isolates from samples collected from hospitals located in Mizoram, India, enterobacteria such as *E. coli*, *K. pneumoniae* and *Salmonella* spp. were isolated. All the isolates were tested for the presence of *blaCTX-M-1* and/or *blaSHV* genes by PCR assay. A total of 54 (13.04%) isolates carried at least one ESBLs genes tested under this study, of which 41 (9.90%) *E. coli*, 11 (2.66%) *K. pneumoniae* and 2 (0.48%) *Salmonella* were found to be positive for *blaCTX-M-1/blaSHV* gene. A total of 4 (10.14%) and 9 (2.17%) isolates were positive for *blaCTX-M-1* and *blaSHV* genes, respectively, whereas, 3 (0.72%) *K. pneumoniae* isolates were positive for both the genes. On the other hand, only 2 (0.48%) *Salmonella* isolates for *blaCTX-M-1* gene (96).

In our study, both rates are lower than those found in the phenotypic profile, however, it must be taken into account that the molecular analysis was performed in only 18 representatives of the studied species, thus presenting a smaller sample than the phenotypic tests. This fact explains why the data from the molecular analyzes are denominated only as potential and also, in addition, the presence of genes in the genome does not necessarily imply phenotypic expression of them (97).

Even molecular analyzes genes are not expressed as host carriers, the only fact of being present in circulating strains is already a high risk, since the onset and spread of the microorganism with drug resistance represents the problem of the interaction of several factors such as an exchange of genetic information between microorganisms, with transfer of genes to new hosts (98).

The presence of PDR profiles in the molecular analyzes and the absence of this profile in the phenotypic analyzes evidences the greater sensitivity of the molecular methodology. The main advantage of qPCR is that it provides a high and fast transfer rate of detection and quantification of target DNA sequences in different matrices. The low amplification time is facilitated by the simultaneous amplification and visualization of the new amplicons formed. However, the mere presence of genes responsible for components of antimicrobial resistance or toxin production does not automatically signify their expression or production (97). Thus, although molecular techniques are very useful, particularly for rapid results, they should be confirmed with standard phenotypic sensitivity tests (99).

Were done the statistic method and according with results of this study the closer to zero, the lower the association level (100). The low linear correlation found in this study ($r^2 = 0.0015$ or $r = 0.038$) should be understood as a comparative analysis of the efficiency of the two methodologies for the detection of antimicrobial resistance. The molecular methodology, PCR, is appreciated due to its high capacity of sensitivity and specificity (101). The low linear correlation found with the Pearson coefficient in this study evidences limitations of the phenotypic methodology and shows greater sensitivity of the molecular methodology for the detection of antimicrobial resistance.

However, it should be borne in mind that the conditions offered in culture media diverge from the actual conditions of a host organism. Since the culture medium is a favorable environment for bacterial growth, it offers optimal conditions for bacterial metabolism, a fact that does not occur in the host organism. This variation of conditions may be determinant for gene regulation, generally leading to the

expression of a greater number of genes in the environment of metabolic stress or gene suppression in environment with favorable growth conditions. This explains why the molecular data found here is compatible with epidemiological data (21).

Conclusion

This study demonstrated that levels of enterobacteria resistant to various antimicrobials are high, both in human and animal clinics. The present antimicrobial resistance study characterized phenotypic and molecular profiles of resistance to beta-lactam antibiotics in enterobacteria. The phenotypic profile was demonstrated by the Antimicrobial Sensitivity Test, performed by plate-diffusion (antibiogram), while the molecular profile was demonstrated from the Molecular Resistance Potential analyzes, which associates data from the literature review with the amplifications by quantitative PCR. MDR, XDR and PDR profiles were found. Being the PDR profile found only by molecular methodology. In this characterization, the detection rate by molecular methodology was higher, demonstrating the greater sensitivity of this technique.

According to the results obtained here, it can be determined that in view of the need for faster diagnosis in emergencies or not, the molecular method, because it is more sensitive, faster and less laborious, can be considered superior to the phenotypic method that presents some limitations such as: reproduction dependence of specific conditions for optimal growth of bacteria, detection of only cultivable organisms, previous preparation of material, greater manipulation and risk of contamination, more time for the final diagnosis.

Declarations

- Ethics and consent to participate

Not applicable.

- Declaration for publication
- Not applicable
- Declaration for availability of data and materials

The authors declare agree to make data and materials available to the BMC journal.

- Declaration of competing interest

The authors declare that the manuscript have no competing of interest in this section.

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- Declaration of authors' contributions

The authors declare the contribution declaration of each authors for the manuscript are:

APS: Has done substantial the acquisition, analysis, interpretation of data; wrote the paper and revised it substantively.

ALS: Has done substantial contribution for the acquisition, analysis, interpretation of data.

CZO: Has done substantial contributions for the conception of the work.

CRMI: Has done substantial for the acquisition of data.

JAA: Has done substantial for the acquisition of data.

MABCJ: Has done substantial for the acquisition of data.

MAP: Has done substantial for the acquisition of data.

MAGA: Has done substantial for the acquisition of data.

IJW: Has done substantial for the acquisition of data.

GPLAG: Has done substantial for the acquisition of data.

ACSS: Has done substantial for the acquisition of data.

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MOS: Has done substantial for the acquisition of data.

CASBB: Has done substantial for the acquisition and analysis of data.

MSB: Has done substantial for the paper review.

LCC: Has done substantial for the acquisition, analysis, interpretation of data; wrote the paper and revised it substantively.

- Declaration of Acknowledgment

Not applicable.

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Tables

Table 1. Oligonucleotides used for amplification of the β -lactam resistance genes of this study.

Genes	Gene sequence from 5' to 3'	Temperature of ringing	Quantity of bases	Access at the GenBank	Amplified fragment Size
<i>blaOXA</i>	Sense: GGCAGCGGGTCCCTTGTC	49,7	19	FN396876.1	171pb
	Reverso: CGATAATGGGCTGCAGCGG	49,7	19		
<i>blaIMP</i>	Sense: CCAGCGTACGGCCACAGA	49,6	19	NG035455.1	138pb
	Reverso: GGTGATGGCTGTTGCGGCA	50,3	19		
<i>blaNDM</i>	Sense: CGGCCGCGTGCTGGTG	49,8	16	JN711113.1	182pb
	Reverso: GGCATAAGTCGCAATCCCCG	50,2	20		
<i>blaSME</i>	Sense: GCGGGCTGCTGTTTTAGAGAGG	50,9	25	KJ188748.1	184pb
	Reverso: TGCAGCAGAAGCCATATCACCTAAT	50,3	22		
<i>blaDHA</i>	Sense: GCGGGCGAATTGCTGCAT	49,8	18	NG041043.1	183pb
	Reverso: TGGGTGCCGGGTAGCG	50,1	17		
<i>blaCMY</i>	Sense: GGATTAGGCTGGGAGATGCTGAA	50,1	23	NG041279.1	158pb
	Reverso: CCAGTGGAGCCCCGTTTTATGC	49,6	21		
<i>blaTEM</i>	Sense: TCCGTGTCGCCCTTATTCCC	49,6	20	KJ923009	165pb
	Reverso: CCTTGAGAGTTTTCGCCCCG	49,6	20		
<i>blaSHV</i>	Sense: GGCAGCGGGTCCCTTGTC	49,7	19	FN396876.1	171pb
	Reverso: CGATAATGGGCTGCAGCGG	49,7	19		
<i>blaVIM</i>	Sense: GTTATGCCGCACCCACCCC	50,3	19	NG036099.1	194 pb
	Reverso: ACCAAACACCATCGGCAATCTG	49,7	22		
<i>blaSPM</i>	Sense: CGAAAATGCTTGATGGGACCG	50,3	21	DQ145284.1	147pb
	Reverso: CACCCGTGCCGTCCAATG	49,7	19		
<i>blaCTX</i>	Sense: CTGAGCTTAGCGGGCCG	50,1	18	FJ815279.1	189pb
	Reverso: AATGGCGGTGTTTAACGTCCG	50,0	21		
<i>blaGIM</i>	Sense: CGGTGGTAACGGCGCAGTG	50,2	19	JX566711.1	149pb
	Reverso: TGCCCTGCTGCGTAACATCG	50,2	20		
<i>blaKPC</i>	Sense: GCGGCTCCATCGGTGTG	49,5	18	AF297554.1	155pb
	Reverso: GTGTCCAGCAAGCCGGCCT	50,4	19		
<i>blaSIM</i>	Sense: GCACCACGGCAAGCGC	50,8	17	EF125010.1	156pb
	Reverso: TGTCTGGCTGCGAACGA	50,0	19		

Table 2 - Percentage of antimicrobial resistance by sample source (%).

Antibiotics	Percentage of antimicrobial resistance (%)					
	Manual Resuscitators	Human cornea	Human tonsils	Veterinary Hospital	Animal bladder	Animal uterus
Ampicillin	42.85	65.21	0.2	90.47	100	100
Aztreonam	0	26.08	0	85.71	0	0
Amoxicillin-clavulanate	0	39.13	0	90.47	100	100
Ceftazidime	100	30.43	0	85.71	100	0
Cefoxitin	42.85	39.13	0.2	76.19	0	0
Cefazolin	0	52.17	100	80.95	100	0
Cefepime	100	30.43	0	95.23	0	0
Ceftriaxone	0	26.08	0	0	0	0
Cefuroxime	100	26.08	0	76.19	0	0
Imipenem	100	30.43	0.2	28.57	100	0
Piperacillin-tazobactam	100	17.39	0	67.66	100	0

Table 3: Bibliographical survey concerning the phenotypic resistance of betalactamases against the corresponding resistance genes. Subtitle: The (+) sign indicates correlation in the literature of the corresponding beta-lactamase coding gene of the column, with the corresponding antibiotic in the horizontal line. While the (-) sign indicates absence of correlation in the gene and antibiotic literature.

Antimicrobials	Genes encoding beta-lactamase enzymes													
	<i>bla</i> _{OXA}	<i>bla</i> _{IMP}	<i>bla</i> _{NDM}	<i>bla</i> _{SME}	<i>bla</i> _{DHA}	<i>bla</i> _{CMY}	<i>bla</i> _{TEM}	<i>bla</i> _{KPC}	<i>bla</i> _{SPM}	<i>bla</i> _{CTX-M}	<i>bla</i> _{VIM}	<i>bla</i> _{SIM}	<i>bla</i> _{GIM}	<i>bla</i> _{SHV}
Beta-lactams														
Ampicillin	-	-	-	+	-	+	-	+	+	-	-	+	-	-
Aztreonam	+	-	+	-	-	-	+	+	+	+	+	+	-	+
Amoxicillin + Clavulanate	-	-	+	-	-	+	+	-	-	+	-	-	-	+
Ceftazidime	+	+	+	-	-	-	+	+	+	+	+	+	-	+
Cefoxitin	-	-	+	-	-	+	+	-	+	+	-	-	-	-
Cefazolin	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Cefepime	+	+	-	-	-	-	+	+	+	+	+	+	-	-
Ceftriaxone	-	-	-	-	-	+	+	+	-	+	-	-	-	+
Cefuroxime	-	-	-	-	-	-	-	+	-	+	+	-	-	+
Imipenem	+	+	-	+	-	-	-	+	+	-	+	+	+	-
Piperacillin + Tazobactam	-	-	-	-	-	+	+	+	+	+	+	+	-	+

Table 4: Rates of detection of phenotypic and molecular antimicrobial resistance.

Antimicrobials	Molecular detection rate (%)	Phenotypic detection rate (%)	Descriptive statistics		
			Standard deviation	Default error	Variance
Ampicillin	83.33	74.28	6.39931637	4.525	40.95125
Aztreonam	94.44	34.28	42.53954396	30.08	1809.6128
Amoxicilina + Clavalunate	88.88	62.85	18.40599	13.015	338.7805
Ceftazidime	94.44	51.42	30.41973	21.51	925.3602
Cefoxitine	94.44	41.42	37.4908	26.51	1405.56
Cefazoline	38.88	54.28	10.88944	7.7	118.58
Cefepime	88.88	44.28	31.53696	22.3	994.58
Ceftriaxone	88.88	41.42	33.55929	23.73	1126.226
Cefuroxime	72.22	8.57	45.00735	31.825	2025.661
Imipenem	88.88	35.71	37.59687	26.585	1413.524
Piperacillin + Tazobactam	94.44	41.42	37.4908	26.51	1405.56

Figures

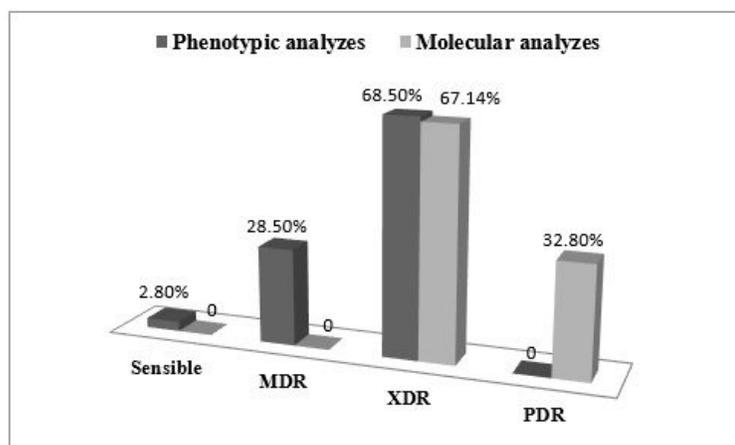


Figure 1

Percentage of Profiles of resistance in Enterobacteriaceae.