Article

Profile of Enterobacteria Resistant to Beta-Lactams

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Abstract: 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 Enterobacteriaceae family. Treatment for bacterial infections is performed with antibiotics, of which the most used are beta-lactams. 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. In this study, 70 exclusive isolates from Brazil were used, half of which were collected in veterinary clinics or hospitals. Phenotypic methodologies were used and real-time PCR was the molecular methodology used, through the Sybr Green system. Regarding the results found in the tests it was observed that 74.28% were resistant to ampicillin, 62.85% were resistant to amoxicillin associated with clavalunate. 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) and the one that was amplified in a smaller number of samples was blaVIM (16.66%). Therefore, high and worrying levels of antimicrobial resistance have been found in enterobacteria, and a way to minimize the accelerated emergence of their resistance includes developing or improving techniques that generate diagnoses with high efficiency and speed.

Keywords: antibiogram; antimicrobial resistance; β-lactams; enterobacterias; molecular diagnosis

1. Introduction

Regarding taxonomy, 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 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 humans and vertebrates gastrointestinal tract [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].

Currently, antimicrobial resistance is one of the most important factors that threaten public health [7]. Transmission between species of resistant bacteria or genetic elements of resistance from animals or the environment to humans has been reported [8,9]. Monitoring hospital environments and those related to animal husbandry and treatment has permanently entered the timeline of the most important studies and annual reports that assess the scope and level of this phenomenon [10].

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 [11]. According to Magiorakos [12], bacteria can be multi drug-resistant (MDR). MDR was classified as having acquired non-susceptibility to at least one agent in three or more antimicrobial categories.

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 [13]. 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 [14].

Beta-lactams are preferred because of their clinical efficacy and safety by virtue of their highly selective toxicity [15]. Resistance to beta-lactams in Enterobacteriaceae and other Gram-negative organisms is primarily mediated by beta-lactamases [16]. Beta-lactamases are enzymes that catalyze the beta-lactam hydrolysis ring leading to antimicrobial inactivation and preventing it from being active against the enzymes responsible for bacterial cell wall synthesis [17].

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system [18]. The antibiogram provides qualitative results by categorizing bacteria as susceptible, intermediate susceptibility 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 [19].

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 the patients to obtain a fast examination result, 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 [20].

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: \(\texttt{bla}\text{SPM}, \texttt{bla}\text{SIM}, \texttt{bla}\text{VIM}, \texttt{bla}\text{KPC}, \texttt{bla}\text{SHV}, \texttt{bla}\text{CTX-M}, \texttt{bla}\text{GIM}, \texttt{bla}\text{OXA}, \texttt{bla}\text{IMP}, \texttt{bla}\text{NDM}, \texttt{bla}\text{SME}, \texttt{bla}\text{DHA}, \texttt{bla}\text{CMY} \text{ and } \texttt{bla}\text{TEM}. \) This study is justified because it is assumed that molecular methods improve accuracy and
efficiency compared to the classical phenotyping method. In addition, it can be released in a short time; helping to improve the effectiveness of antibiotic therapy.

2. 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 the human clinic, 20% from an animal clinic, 10% from a human hospital environment and 30% from the veterinary hospital environment.

This antimicrobial resistance study 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 clavulinate, 38 (54.28%) were resistant to cefazolin, and 6 (8.57%) were resistant to cefuroxime. Table 1 shows the percentage of antimicrobial resistance by sample source of Enterobacteriaceae.

Table 1. Percentage of antimicrobial resistance by sample source (%).

| Antibiotics | 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 |
| Cefazidine  | 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 |

Phenotypically, using the antibiogram method, Enterobacter aerogenes, Enterobacter agglomerans and Cedecea neteri species stand out, which have the highest resistance rate, being resistant to 10 of the 11 tested antibiotics (90.9%).

The sensible and resistance profiles, found phenotypically in this study, determined that among the enterobacteria studied here there was a predominance of 2.8% sensible and 97.2% resistance profiles. Among the resistance profiles, potential MDR profiles were also researched and ESBL, AmpC, MBL, Carbapenemases and CRE were quantified. These data can be observed in Figure 1.

Phenotypic analyzes revealed that 28.5% of the total bacteria studied are MDR. Still on the phenotypic profiles, the penicillin group was the antibiotic for which there was the highest resistance rate. There was resistance in at least one of the studied penicillins, in approximately 85.71% of the bacteria. For cephalosporins, there was resistance to at least one of those tested in 77.14% of bacteria, a relatively high number of which shows that these 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 determining the CRE profile. 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 species E. agglomerans showed phenotypic resistance data with profiles sensitive, MDR and CRE. This shows that within the same bacterial species the resistance possibilities are very variable. The species Yersinia ruckeri, was the one that presented more number of resistance mechanisms (ESBL, MBL and AmpC). The species that did not present any of the mechanisms were: Escherichia blattae, Hafnia alvei, Raoultella terrigena and Citrobacter freundii.
nicillins, in approximately 85.71% of the, stood out showing the highest resistance rate. The antibiotic that presented the lowest percentage of resistance was the monobactam aztreonam. 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 blaIMP gene, 44.44% for the blaSHV and blaSPM genes, 38.88% for the blaKPC gene, 33.33% for the blaSME gene and 16.66% for the blaVIM gene.

The phenotypic profile of resistance to beta-lactam antimicrobials was determined in 19 bacterial species among enterobactérias: regarding 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%). For methodological reasons the resistance mechanisms ESBL, AmpC, MBL and Carbapenemases have not been studied molecularly, only CRE. The molecular profile of resistance to beta-lactam antimicrobials was determined in 18 bacterial species among enterobacteria: 94.44% showing resistance for aztreonam, ceftazidima, cefoxitin and pipercillin associated with beta-lactamase inhibitor tazobactam. Phenotypic and molecular data are compared in Table 2.

### Table 2. Rates of detection of phenotypic and molecular antimicrobial resistance.

| Antimicrobials           | Molecular Detection Rate (%) | Phenotypic Detection Rate (%) | Descriptive Statistics |
|--------------------------|------------------------------|-------------------------------|------------------------|
|                          | Standard Deviation          | Default Error                 | Variance               |
| Amoxicilina + Clavulinate| 83.33                        | 74.28                         | 6.39931637             | 4.525                  | 40.95125               |
| DHA                      | 34.34                        | 34.28                         | 42.53954396            | 30.08                  | 1809.6128              |
| Pipercillin + Tazobactam | 94.44                        | 93.92                         | 10.88944               | 7.7                    | 118.58                 |
| Carbenemase               | 61.11                        | 60.75                         | 33.5929                | 23.73                  | 1126.226               |
| AmpC                     | 66.66                        | 65.32                         | 37.4908                | 26.51                  | 1405.56                |
| MBL                      | 88.88                        | 88.35                         | 30.41973               | 21.51                  | 925.3602               |
| ESBL                     | 94.44                        | 94.28                         | 37.4908                | 26.51                  | 1405.56                |
| SIM                      | 38.88                        | 38.42                         | 37.4908                | 26.51                  | 1405.56                |
| SME                      | 88.88                        | 88.52                         | 37.4908                | 26.51                  | 1405.56                |
| IMP                      | 94.44                        | 94.31                         | 37.4908                | 26.51                  | 1405.56                |
| Ceftriaxime              | 94.44                        | 94.31                         | 37.4908                | 26.51                  | 1405.56                |
| Cefoxilina               | 94.44                        | 94.31                         | 37.4908                | 26.51                  | 1405.56                |
| Cefpime                  | 88.88                        | 88.52                         | 37.4908                | 26.51                  | 1405.56                |
| Ceftriaxone              | 88.88                        | 88.52                         | 37.4908                | 26.51                  | 1405.56                |
| Cefuroxime               | 72.22                        | 71.86                         | 37.4908                | 26.51                  | 1405.56                |
| Amoxicilina + Clavulinate| 88.88                        | 88.52                         | 37.4908                | 26.51                  | 1405.56                |
| Cephalosporins           | 94.44                        | 94.31                         | 37.4908                | 26.51                  | 1405.56                |

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 resistance to a greater number of antibiotics were: E aerogenes, R. terrigena, Morganella morganii, Edwardsiella ictaluri, C. neteri, Salmonella paratyphi and Y. ruckeri., exhibiting resistance potential for all antibiotics (100%) tested. C. freundii, Klebsiella spp., E. coli, E blattae,
Providencia rustigiani and Erwinia persicina were studied, showing potential resistance in 10 among 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 MDR and CRE data were, respectively, 100% and 88.88% among the studied species. These data can be observed in Figure 1.

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.

In this study, the detection rate of antimicrobial resistance by molecular methodology was generally higher than the detection rate by phenotypic methodology. This study showed that the presence of the resistance gene in the bacterial genome does not necessarily imply its expression, therefore it is necessary to develop the phenotypic methodology.

An experiment was carried out to verify the plasmid profile and from plasmid DNA digested were no identified sites to EcoR 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.

3. 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 [21].

Except for C. neteri, which is a strain of animal origin, these bacterial species are in accordance with epidemiological data indicated by ANVISA [22], 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.

Regarding multidrug resistance (MDR) and carbapenem-resistant Enterobacteriaceae (CRE) profiles have recently been updated by the Center for Disease and Control and European Center for Diseases Control and Prevention, promoted aiming at the international standardization of these terminologies, as published by Magiorakos et al, [12], in which, MDR was defined as the resistance to at least one agent in three or more categories of antimicrobials.

According to the authors, Enterobacteria resistant to carbapenems infections are associated with high mortality rates (up to 70%), making them particularly challenging from a clinical standpoint [11].

Logan and Weinsteins [21] showed a global distribution of carbapenemase degenerations in Enterobacteriaceae. Carbapenem-resistant enterobacteria have emerged as a major cause of nosocomial infections worldwide and are characterized by rapid and progressive dissemination [23].

In a study of 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, carbapenem minimum inhibitory concentration (MICs) for the CRE card ranged from ≤1 to 265 >8 µg/mL for imipenem, which also demonstrated higher resistance to this antimicrobial [24].

A study carried out in northeastern Brazil showed that in 672 positive urocultures for urinary tract infection, the etiological agent belonged to the Enterobacteriaceae family in 86.9%, and among them 29 (4.8%) were ESBL [25].

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 [3,6,16,26–117].
Table 3. Bibliographical survey concerning the phenotypic resistance of beta-lactamases against the corresponding resistance genes. subtitle: the (+) sign indicates a 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 an absence of correlation in the gene and antibiotic literature.

| Antibiotics                  | bla_{OXA} | bla_{IMP} | bla_{NDM} | bla_{SME} | bla_{DHA} | bla_{CMY} | bla_{TEM} | bla_{KPC} | bla_{SFM} | bla_{CTX-M} | bla_{VIM} | bla_{SIM} | bla_{GIM} | bla_{SHV} |
|------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-------------|-----------|-----------|-----------|-----------|
| Ampicillin                   | +         | -         | -         | +         | -         | +         | +         | +         | +         | +           | -         | +         | -         | +         |
| Aztreonam                    | +         | +         | +         | -         | +         | +         | +         | +         | +         | +           | +         | -         | +         | +         |
| Amoxicillin + Clavulanate    | +         | +         | +         | -         | +         | +         | +         | -         | +         | +           | -         | +         | -         | +         |
| Cefazidime                   | +         | +         | +         | -         | +         | +         | +         | +         | +         | +           | -         | -         | +         | +         |
| Cefoxitin                    | +         | -         | +         | -         | +         | +         | +         | +         | +         | +           | -         | -         | +         | +         |
| Cefazolin                    | +         | +         | +         | -         | +         | +         | +         | -         | +         | +           | -         | -         | +         | +         |
| Cefepime                     | +         | +         | +         | -         | +         | +         | +         | +         | +         | +           | +         | +         | -         | +         |
| Ceftriaxone                  | +         | +         | +         | -         | +         | +         | +         | -         | +         | +           | -         | -         | +         | +         |
| Imipenem                     | +         | +         | +         | +         | +         | +         | +         | +         | +         | +           | +         | +         | +         | +         |
| Piperacillin + Tazobactam    | +         | +         | +         | -         | +         | +         | +         | +         | +         | +           | +         | +         | +         | -         |
The data from the bibliographic review carried out here show that the beta-lactamases most frequently correlated with beta-lactams are *bla*OXA, *bla*TEM, *bla*KPC, *bla*CTX-M and *bla*SHV. However, among the total genes found in this study, the ones with the highest percentage were *bla*GIM and *bla*SIM, both with 66.66%. In the literature review, the *bla*GIM gene was correlated only with beta-lactam imipenem, while *bla*SIM was correlated with amoxicillin, aztreonam, ceftazidime, cephepine, imipenem and piperacillin + tazobactam. Resistance to beta-lactam imipenem gives the bacteria the CRE profile, and although phenotypic analyzes show a low detection rate of imipenem (35.71%), the molecular detection rate of imipenem was the second-highest detection rate with a value of 88.88%.

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 *bla*KPC, *bla*OXA-48, *bla*NDM, *bla*SPM, *bla*IMP, *bla*VIM and *bla*GIM genes were investigated by PCR and multiplex PCR. About the number of studied microorganisms, the genotypic tests evidenced that *bla*KPC was the most prevalent gene, in 31% ($n = 10$) of the samples, followed by *bla*IMP, in 12.5% ($n = 4$) [118].

In a study conducted in eight hospitals in Paris surroundings, France, twelve isolates were collected in twelve patients, 11 *Klebsiella pneumoniae* and 1 *Klebsiella oxytoca*. All isolates showed *bla*DHA gene and (4/12) 33.33% *bla*TEM gene [119].

In another study, 88 phenotypically ESBLs positive isolates 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 *bla*CTX-M-1 and/or *bla*SHV 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 *bla*CTX-M-1 or *bla*SHV gene. A total of 4 (10.14%) and 9 (2.17%) isolates were positive for *bla*CTX-M-1 and *bla*SHV 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 *bla*CTX-M-1 gene [120].

In our study, both rates are lower than those found in the phenotypic profile, however, it should be considered 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 [121].

Even molecular analyzes genes are not expressed as host carriers and 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 shows the problem of the interaction of several factors such as an exchange of genetic information between microorganisms, through the transfer of genes to new hosts [32].

The presence of more resistance profiles in molecular analyzes than in phenotypic analyzes testify the greater sensitivity of the molecular methodology. qPCR provides a high advantage of fast transferring detection rate 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 [34]. Thus, although molecular techniques are very useful, particularly for rapid results, they should be confirmed with standard phenotypic sensitivity tests [122].

The statistical method was performed [123], and according to the low linear correlation found in this study ($r^2 = 0.0015$ or $r = 0.038$), it should be known 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 [124]. 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 bear in mind the conditions offered in the growth medium diverge from the actual conditions of a host organism. Since the growth 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 an environment with favorable growth conditions. This explains why the molecular data found here is compatible with epidemiological data [125].

4. 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 terrigena*, *Escherichia coli*, *Escherichia blattae*, *Edwardsiella ictaluri*, *Cedecea neteri*, *Erwinia persicina*, *Providencia rustigiani*, *Salmonella paratyphi*, *Salmonella typhi*, *Yersinia ruckeri*, *Serratia marceccens* and *Hafnia alvei* (Table 4). Bacteria came 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 were 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 in a 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.

| Bacterial Genus         | Number of Samples |
|-------------------------|-------------------|
| *Cedecea neteri*        | 2                 |
| *Citrobacter freundii*  | 3                 |
| *Edwardsiella ictaluri* | 1                 |
| *Enterobacter aerogenes*| 12                |
| *Enterobacter agglomerans* | 6               |
| *Erwinia persicina*     | 1                 |
| *Escherichia blattae*   | 1                 |
| *Escherichia coli*      | 12                |
| *Hafnia alvei*          | 3                 |
| *Klebsiella spp.*       | 7                 |
| *Morganella morganii*   | 2                 |
| *Proteus mirabilis*     | 4                 |
| *Providencia rustigiani*| 1                 |
| *Providencia spp.*      | 1                 |
| *Raoultella terrigena*  | 1                 |
| *Salmonella paratyphi*  | 1                 |
| *Salmonella spp.*       | 2                 |
| *Salmonella typhi*      | 2                 |
| *Serratia marceccens*   | 6                 |
| *Yersinia ruckeri*      | 1                 |
| *Yersinia spp.*         | 1                 |

After being stored as a biorepository, 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, amoxiline-clavulanate 20/10 µg, aztreonam 30 µg, cefazolin 30 µg, cefepime 30 µg, cefoxitin 30 µg, cefuroxime 30 µg, cefazidime 30 µg; ceftriaxone 30, imipen 10 µg and piperacillin-tazobactam 100/10 µg. The quality control procedure was followed, strains *E. coli* ATCC® 35,218 were used for combinations of β-lactam inhibitors/β-lactamases [126].
For the phenotypic detection of extended spectrum beta-lactamases (ESBL) production, from enterobacteria of this study, the statistic method was performed [126] and according to the results low linear correlation was found ($r^2 = 0.0015$ or $r = 0.038$), and it is important to recognize that the data presented good efficiency in both methodologies for the detection of antimicrobial resistance.

For the AmpC-type beta-lactamase phenotypic detection, the induction test was performed using antimicrobial susceptibility testing, performed by the disk diffusion assay (Kirby–Bauer technique) according to the 2015 European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations [127].

Imipenem and meropenem discs were used for the carbapenemases phenotypic investigation and interpretation of the sensitivity following the criteria established by CLSI [128]. At least one bacterium that showed resistance of the carbapenemases was submitted to the MBL screening test, using the enzyme blockade method and following the recommendations of ANVISA [22]. The test used imipenem (10 µg) and meropenem (10 µg) disc, positioned parallel to two other imipenem and meropenem discs added with 10 µL 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-Hintos 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 [128].

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 5). Reactions were prepared using the Sybr Green (Sybr Green qPCR master mix LOW ROX-100 reactions $\times$ 25 µL) real-time PCR kit, following the 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 EcoRI for enzyme EcoRI and Buffer V2 for Hind III); 1 µL of EcoRI or Hind III enzyme (10 U/µL) (Ludwigbiotec), 15 µL H2O; 2 µL template DNA (~300 ng/µL). The tubes were placed in thermo-blocks 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.
| Genes | Gene Sequence from 5’ to 3’ | Temperature of Ringing | Quantity of Bases | Access at the GenBank | Amplified Fragment Size |
|-------|----------------------------|------------------------|------------------|----------------------|------------------------|
| blaOXA | Sense: GGCAGCGGGTTCCTTGGTC | 49.7                   | 19               | FN396876.1           | 171pb                  |
|       | Reverso: CGATAATGGGCTGCAGCGG | 49.7                   | 19               |                      |                        |
| blaIMP | Sense: CGAGCTACGGCCACAGA     | 49.6                   | 19               | NG035455.1           | 138pb                  |
|       | Reverso: GGTGATGCTGCTGGCGCA | 50.3                   | 19               |                      |                        |
| blaNDM | Sense: CGCCCGCGCTGCTGTTG   | 49.8                   | 16               | JN711113.1           | 182pb                  |
|       | Reverso: GGCGGCTGCTGTTTAAGAGG | 50.9                   | 25               |                      |                        |
| blaSME | Sense: GGCACAGAAAGCCTACTCAAT | 50.3                   | 22               | KJ188748.1           | 184pb                  |
|       | Reverso: TGGTGCAGCCGGAAGCGC | 50.1                   | 17               |                      |                        |
| blaCMY | Sense: GATAGCGTGGAGATGTGCAAA | 50.1                   | 23               | NG041279.1           | 158pb                  |
|       | Reverso: CGAGTGGACCGCGTTTATG | 49.6                   | 21               |                      |                        |
| blaTEM | Sense: TCCGTGTCCGCCCTTATTCC | 49.6                   | 20               | KJ923009             | 165pb                  |
|       | Reverso: CCTTGAAGTTTTTCGCCCCG | 49.6                   | 20               |                      |                        |
| blaSHV | Sense: GGCGGCGCGGCTCCGTCG   | 49.7                   | 19               | FN396876.1           | 171pb                  |
|       | Reverso: CGATAATGCGCTGACGGG | 49.7                   | 19               |                      |                        |
| blaDHA | Sense: GTTATGCGGCCAACCACCC | 50.1                   | 19               | NG041043.1           | 183pb                  |
|       | Reverso: ACCACACCATCGCGCAATCTG | 49.7                   | 22               |                      |                        |
| blaSPM | Sense: CGAAAATGCTTTGATGGGACCG | 50.3                   | 21               | DQ145284.1           | 147pb                  |
|       | Reverso: CACCCGTGCGCTCAAATGT | 49.7                   | 19               |                      |                        |
| blaCTX | Sense: CTGAGCTAGCGCGCGGCGG | 50.1                   | 18               | FJ815279.1           | 189pb                  |
|       | Reverso: AATGCGCGGTGTAAAGCTGG | 50.0                   | 21               |                      |                        |
| blaGIM | Sense: CCCTGGTTAAGCGCGCTGTG | 50.2                   | 19               | JX566711.1           | 149pb                  |
|       | Reverso: TGCCCTGCTGTAACCATCG | 50.2                   | 20               |                      |                        |
| blaKPC | Sense: GGCGGCTCCACTGGTGTG  | 49.5                   | 18               | AF297554.1           | 155pb                  |
|       | Reverso: GTGTCGACAGCAAGCCTCAC | 50.4                   | 19               |                      |                        |
| blaSIM | Sense: GCACCCCGGCAAGCGC     | 50.8                   | 17               | EF125010.1           | 156pb                  |
|       | Reverso: TGTCCTGGCTGCGAAGCA | 50.0                   | 19               |                      |                        |
5. Conclusions

This study demonstrated high resistance levels of enterobacteria to various antimicrobials, both in humans and animals. 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 to the amplification by quantitative PCR. MDR and CRE profiles were found. 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, given the need for faster diagnosis in emergencies or not, the molecular method, because its more sensitive, faster and less laborious process, can be considered superior to the phenotypic method in which has some limitations such as dependence on specific conditions of reproduction for the ideal growth of bacteria, detection of only cultivable organisms, previous preparation of the material, greater manipulation and risk of contamination, and longer time for the final diagnosis.

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References

1. Mlaga, K.D.; Lotte, R.; Montaudié, H.; Rolain, J.-M.; Ruimy, R. 'Nissabacter archeti’ gen. nov., sp. nov., a new member of Enterobacteriaceae family, isolated from human sample at Archet 2 Hospital, Nice, France. New Microbes New Infect. 2017, 17, 81–83. [CrossRef] [PubMed]
2. Winn, W.C.; Allen, S.D.; Janda, W.M.; Koneman, S. Diagnóstico Microbiológico: Texto e Atlas colorido. Guanab. Koogan. 2008, 16, 1–1760.
3. Coque, T.; Baqueiro, F.; Canton, R. Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Eurosurveillance 2008, 13, 1–11.
4. Lutgring, J.D.; Limbagob, B.M. The Problem of Carbapenemase-Producing-Carbapenem-Resistant Enterobacteriaceae Detection. J. Clin. Microbiol. 2016, 54, 529–534. [CrossRef] [PubMed]
5. Vega, S.; Dowzicky, M.J. Antimicrobial susceptibility among Gram-positive and Gram-negative organisms collected from the Latin American region between 2004 and 2015 as part of the Tigecycline Evaluation and Surveillance Trial. Ann. Clin. Microbiol. Antimicrob. 2017, 16, 1–16. [CrossRef]
6. Ozsurekci, Y.; Aykac, K.; Cengiz, A.B.; TanrıBaşaranoglu, S.; Sancak, B.; Karahan, S.; Kara, A.; Ceyhan, M. Bloodstream infections in children caused by carbapenem-resistant versus carbapenem-susceptible gram-negative microorganisms: Risk factors and outcome. Diagn. Microbiol. Infect. Dis. 2017, 87, 359–364. [CrossRef]
7. Prestinaci, F.; Pezzotti, P.; Pantosti, A. Antimicrobial resistance: A global multifaceted phenomenon. Pathog. Glob. Health 2015, 109, 309–318. [CrossRef]
8. Tang, L.K.; Caffrey, P.N.; Nóbrega, B.D.; Cork, C.S.; Ronksley, E.P.; Barkema, W.H.; Polachek, J.A.; Ganshorn, H.; Sharma, N.; Kellner, D.J.; et al. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: A systematic review and meta-analysis. Lancet Planet. Health 2017, 1, 316–327. [CrossRef]
9. Bougnom, P.B.; Piddock, J.L. Wastewater for urban agriculture. A significant factor in dissemination of antibiotic resistance. Environ. Sci. Technol. 2017, 51, 5863–5864. [CrossRef]
10. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. *EFSA J.* 2019, 17, 278. [CrossRef]

11. Rossoline, G.M.; Arena, F.; Pecile, P.; Pollini, S. Update on the antibiotic resistance crisis. *Curr. Opin. Pharmacol.* 2014, 18, 56–60. [CrossRef] [PubMed]

12. Magiorakos, A.-P.; Srinivasan, A.; Carey, R.; Carmeli, Y.; Falagas, M.E.; Giske, C.; Harbarth, S.; Hindler, J.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Microbiology* 2011, 18, 268–281.

13. Levy, S.B.; Marshall, B. Antibacterial resistance worldwide: Causes, challenges and responses. *Nat. Med. Suppl.* 2004, 10, 122–129. [CrossRef] [PubMed]

14. Koraimann, G. Spread and Persistence of Virulence and Antibiotic Resistance Genes: A Ride on the F Plasmid Conjugation Module. *EcoSal Plus* 2018, 8, 1–23. [CrossRef] [PubMed]

15. Chant, C.; Leung, A.; Friedrich, J.O. Optimal dosing of antibiotics in critically ill patients by using continuous/extended infusions: A systematic review and metaanalysis. *Crit. Care* 2013, 17, R279. [CrossRef] [PubMed]

16. Martinez-Martinez, L.; González-López, J.J. Carbapenemases in Enterobacteriaceae: Types and molecular epidemiology. *Enferm. Infec. y Microbiol. Clin.* 2014, 32, 4–9. [CrossRef]

17. Bertonchel, C.D.M.; Hörner, R. Uma revisão sobre metalo-β-lactamases. *Rev. Bras. de Ciências Farm.* 2008, 44, 577–599. [CrossRef]

18. Lekshmi, P.N.C.J.; Sumi, B.; Viveka, S.; Jeeva, S.; Brindha, R. Antibacterial activity of nanoparticles from *Allium* sp. *J. Microbiol. Biotechnol.* 2012, 2, 115–119.

19. Balouiri, M.S.; Moulay, I.; Saad, K. Methods for in vitro evaluating antimicrobial activity: A review. *J. Pharm. Anal.* 2016, 6, 71–79. [CrossRef]

20. Tuite, N.; Reddington, K.; Barry, T.; Zumla, A.; Enne, V. Rapid nucleic acid diagnostics for the detection of antimicrobial resistance in Gram-negative bacteria: Is it time for a paradigm shift? *J. Antimicrob. Chemother.* 2014, 69, 1729–1733. [CrossRef]

21. Jabur, A.P.L.; Magalhães, L.G.; Borges, A.A.; Cardoso, A.L. Infecção urinária em gestantes atendidas em um laboratório de análises clínicas de Goiânia-GO entre 2012 e 2013. *Estudos* 2014, 41, 637–641.

22. Agência Nacional de Vigilância Sanitária. Boletim de Segurança do Paciente e Qualidade em Serviços de Saúde n° 16 (Corrigido). 2017. Available online: https://www20.anvisa.gov.br/segurancadopaciente/index.php/publicacoesitem/boletim-seguranca-do-paciente-e-qualidade-em-servicos-de-saude-n-16-avaliacao-dos-indicadores-nacionais-das-infeccoes-relacionadas-a-assistencia-a-saude-iras-e-resistencia-microbiana-do-ano-de-2016 (accessed on 19 September 2018).

23. Logan, L.K.; Weinstein, R.A. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. *J. Infect. Dis.* 2017, 215, S28–S36. [CrossRef] [PubMed]

24. Secchyna, F.; Gaur, R.; Sandlund, J.; Truong, C.; Tremintin, G.; Kältz, D.; Gomez, C.; Tamburini, F.B.; Andermann, T.M.; Bhatt, A.; et al. Diverse Mechanisms of Resistance in Carbapenem-Resistant Enterobacteriaceae at a Health Care System in Silicon Valley, California. *BioRxiv* 2018. [CrossRef]

25. Neto, M.A.R.; Rios, V.M.; Corá, L.F.; Fonseca, M.M.; Ferreira-Paim, K.; Fonseca, F.M. High rates of antimicrobial resistance of ESBL-producing Enterobacteriaceae isolated from clinical samples in Northeast of Brazil. *Infec. Dis.* 2018, 50, 229–231. [CrossRef]

26. Agência Nacional de Vigilância Sanitária. *Nota Técnica N° 01/2013:Medidas de Prevenção e Controle de Infeccões por Enterobactérias Multirresistentes;* Agência Nacional de Vigilância Sanitária: Brasilia, Brazil, 2013.

27. Wendel, A.F.; Brodner, A.H.B.; Wydra, S.; Ressina, S.; Henrich, B.; Pfeifer, K.; Tolemen, M.A.; MacKenziea, C.R. Genetic Characterization and Emergence of the Metallo- β-Lactamase GIM-1 in *Pseudomonas* spp. and Enterobacteriaceae during a Long-Term Outbreak. *Antimicrob. Agents Chemother.* 2013, 57, 5162–5165. [CrossRef]

28. Pfeifer, Y.; Cullik, A.; Witte, W. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. *Int. J. Med Microbiol.* 2010, 300, 371–379. [CrossRef]

29. Verdut, C.; Benzerara, Y.; Gautier, V.; Adam, O.; Ould-Hocine, Z.; Arlet, G. Emergence of DHA-1-Producing *Klebsiella* spp. in the Parisian Region: Genetic Organization of the ampC and ampR Genes Originating from Morganella morganii. *Antimicrob. Agents Chemother.* 2006, 50, 607–617. [CrossRef]
30. Kjeldsen, T.S.B.; Overgaard, M.; Nielsen, S.S.; Bortolaia, V.; Jelsbak, L.; Sommer, M.; Guardabassi, L.; Olsen, J.E. CTX-M-1 β-lactamase expression in *Escherichia coli* is dependent on cefotaxime concentration, growth phase and gene location. *J. Antimicrob. Chemother.* 2015, 70, 62–70. [CrossRef]

31. Warjri, I.; Dutta, T.K.; Laizampuia, H.; Chandra, R. Detection and characterization of extended-spectrum β-lactamases (blaCTX-M-1 and blaSHV) producing *Escherichia coli*, *Salmonella* spp. and *Klebsiella pneumoniae* isolated from humans in Mizoram. *Vet. World* 2015, 8, 599–604. [CrossRef]

32. Machuca, J.; Agüero, J.; Mírő, B.; Conejob, M.C.; Oteob, J.; Boub, G.; González-López, J.J.; Oliverb, A.; Navarrob, F.; Pacasual, A.; et al. Prevalencia en España de mecanismos de resistencia a quinolonas enterobacterias productoras de betalactamasas de clase C adquiridas/o carbapenemasas. *Enferm. Infect. y Microbiol. Clinica* 2017, 35, 487–492. [CrossRef]

33. Pietscha, M.; Eller, C.; Wendtc, C.; Holfelderc, M.; Falgenhauerd, L.; Fruthe, A.; Grösslaf, T.; Leistnerf, R.; Valenzag, G.; Wernera, G.; et al. Molecular characterisation of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates from hospital and ambulatory patients in Germany. *Vet. Microbiol.* 2017, 200, 130–137. [CrossRef] [PubMed]

34. Wang, L.; Liu, P.; Wei, D.; Liu, Y.; Wan, L.; Xiang, T.; Zhang, Y. Clinical isolates of uropathogenic *Escherichia coli* ST131 producing NDM-7 metallo-β-lactamase in China. *Int. J. Antimicrob. Agents* 2016, 48, 41–45. [CrossRef] [PubMed]

35. Bush, K. A resurgence of -lactamate inhibitor combinations effective against multidrug-resistant Gram-negative pathogens. *Int. J. Antimicrob. Agents* 2015, 46, 483–493. [CrossRef] [PubMed]

36. Yang, H.; Chen, H.; Yang, Q.; Chen, M.; Wang, H. High Prevalence of Plasmid-Mediated Quinolone Resistance Genes *qnr* and *aac(6)-Ib-cr* in Clinical Isolates of Enterobacteriaceae from Nine Teaching Hospitals in China. *Antimicrob. Agents Chemother.* 2008, 52, 4268–4273. [CrossRef] [PubMed]

37. Yong, D.; Telemab, M.A.; Giske, C.G.; Cho, H.S.; Sundamb, K.; Lee, K.; Walsh, T.R. Characterization of a New Metallo–Lactamase Gene, blaNDM-1, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in *Klebsiella pneumoniae* Sequence Type 14 from India. *Antimicrob. Agents Chemother.* 2009, 53, 5046–5054. [CrossRef]

38. Sampaio, J.L.M.; Gales, A.C. Antimicrobial resistance in Enterobacteriaceae in Brazil: Focus on β-lactams and polymyxins. *Braz. J. Microbiol.* 2016, 47, 31–37. [CrossRef]

39. Singh-Moodley, A.; Perovic, O. Antimicrobial susceptibility testing in predicting the presence of carbapenemase genes in Enterobacteriaceae in South Africa. *BioMed Central. Infect. Dis.* 2016, 16, 1–10. [CrossRef]

40. Magagnin, C.M.; Rozales1, F.P.; Antochevis, L.; Nunes, L.S.; Martins, A.S.; Barth, A.L.; Sampaio, J.M.; Zavascki, A.P. Dissemination of *bla*OXA-370 gene among several Enterobacteriaceae species in Brazil. *Eur. J. Clin. Microbiol. Infect. Dis.* 2017, 36, 1907–1910. [CrossRef]

41. Kayama, S.; Ohge, H.; Sugai, M. Rapid discrimination of *bla*IMP-1, *bla*IMP-6, and *bla*IMP-34 using a multiplex PCR. *J. Microbiol. Methods* 2017, 135, 8–10. [CrossRef]

42. Duin, D.V.; Doi, Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. *Virulence* 2017, 8, 460–469. [CrossRef]

43. Jeong, S.H.; Kim, H.; Kim, J.; Shin, D.H.; Kim, H.S.; Park, M.; Shin, S.; Hong, J.S.; Lee, S.S.; Song, W. Prevalence and Molecular Characteristics of Carbapenemase-Producing Enterobacteriaceae From Five Hospitals in Korea. *Ann. Lab. Med.* 2016, 36, 529–535. [CrossRef]

44. Ingól, B.; Paul, D.; Maurya, A.P.; Bora, D.; Chanda, D.D.; Chakravarty, A.; Bhattacharjee, A. Occurrence of *bla*DHA-1 mediated cephalosporin resistance in *Escherichia coli* and their transcriptional response against cephalosporin stress: A report from India. *Ann. Clin. Microbiol. Antimicrob.* 2017, 16, 1–8. [CrossRef]

45. Srirattakarn, A.; Lulitanond, A.; Wilailuckana, C.; Charoensri, N.; Wonglakorn, L.; Saenjamla, P.; Chaimane, P.; Daduang, J.; Chanawong, A. Rapid and simple identification of carbapenemase genes, *blaNDM*, *bla*OXA-48, *bla*VIM, *bla*IMP-14 and *bla*KPC groups, in Gram-negative bacilli by in-house loop-mediated isothermal amplification with hydroxynaphthol blue dye. *World J. Microbiol. Biotechnol.* 2017, 33, 130–140. [CrossRef] [PubMed]

46. Aayla, A.T.; Ácuña, H.M.B.; Calvo, M.T.A.; Morales, J.L.V.; Chacón, E.C. Emergencia de β-lactama AmpC plasmidica del grupo CMY-2 en *Shigella sonnei* y *Salmonella* spp. en Costa Rica, 2003–2015. *Pan Am. J. Public Health* 2016, 40, 70–75.
47. Suwantarat, N.; Logan, L.K.; Carroll, K.C.; Bonomo, R.A.; Simner, P.J.; Rudin, S.D.; Milstone, A.M.; Tekle, T.; Ross, T.; Tamma, P.D. The Prevalence and Molecular Epidemiology of Multidrug-Resistant Enterobacteriaceae Colonization in a Pediatric Intensive Care Unit. *Infect. Control Hosp. Epidemiol.* 2016, 37, 535–543. [CrossRef] [PubMed]
48. Silva, K.C.; Linçopan, N. Epidemiologia das betalactamases de espectro estendido no Brasil: Impacto clínico e implicações para o agronegócio. *J. Bras. de Patol. e Med. Lab.* 2012, 48, 91–99. [CrossRef]
49. Li, X.; Pletsiat, P.; Nikaidoc, H. The Challenge of Efflux-Mediated Antibiotic Resistance in Gram-Negative Bacteria. *Clin. Microbiol. Rev.* 2015, 28, 337–418. [CrossRef]
50. Bialvaei, A.Z.; Kafil, H.S.; Asgharzadeh, M.; Memar, M.Y.; Yousefi, M. Current methods for the identification of carbapenemases. *J. Chemother.* 2016, 28, 1–19. [CrossRef]
51. Xia, J.; Gao, J.; Tang, W. Nosocomial infection and its molecular mechanisms of antibiotic resistance. *Biosci. Trends.* 2016, 10, 14–21. [CrossRef]
52. Jacoby, G.A. AmpC Beta-Lactamases. *Clin. Microbiol. Rev.* 2009, 22, 161–182. [CrossRef]
53. Rood, I.G.H.; Li, Q. Review: Molecular detection of extended spectrum-β-lactamase- and carbapenemase-producing Enterobacteriaceae in a clinical setting. *Diagn. Microbiol. Infect. Dis.* 2017, 89, 245–250. [CrossRef] [PubMed]
54. Jones-Dias, D.; Manageiro, V.; Caniça, M. Influence of agricultural practice on mobile bla genes: IncI1-bearing CTX-M, SHV, CMY and TEM in *Escherichia coli* from intensive farming soils. *Environ. Microbiol.* 2016, 18, 260–272. [CrossRef]
55. Bush, K. Overcoming β-lactam resistance in Gram-negative pathogens. *Future Med. Chem.* 2016, 8, 921–924. [CrossRef]
56. Bush, K.; Courvalin, P.; Dantas, G.; Davies, J.; Eisenstein, B.; Huovinen, P.; Jacoby, G.A.; Kishony, R.; Kreiswirth, B.N.; Kutter, E.; et al. Tackling antibiotic resistance. *Nat. Rev.* 2011, 9, 895–896. [CrossRef]
57. Bush, K.; Jacoby, G.A. Updated Functional Classification of B-Lactamases. *Future Med. Chem.* 2016, 8, 921–924. [CrossRef] [PubMed]
58. Bush, K. The ABCD’s of β-lactamase nomenclature. *J. Infect. Chemother.* 2013, 19, 549–559. [CrossRef] [PubMed]
59. Page, M.G.P.; Bush, K. Discovery and development of new antibacterial agents targeting Gram-negative bacteria in the era of pandrug resistance: Is the future promising? *ScienceDirect* 2014, 18, 91–97. [CrossRef] [PubMed]
60. Wei, D.; Wan, L.; Yu, Y.; Xu, Q.; Deng, Q.; Cao, X.; Liu, Y. Characterization of Extended-Spectrum β-Lactamase, Carbapenemase, and Plasmid Quinolone Determinants in *Klebsiella pneumoniae* Isolates Carrying Distinct Types of 16S rRNA Methylase Genes, and Their Association with Mobile Genetic Elements. *Microb. Drug Resist.* 2015, 21, 186–193. [CrossRef]
61. Rocha, D.A.C.; Campos, J.C.; Passadore, L.F.; Sampaio, S.C.F.; Nicolpho, A.C.; Sampaio, J.L.M. Frequency of Plasmid-Mediated AmpC β-Lactamases in *Escherichia coli* Isolates from Urine Samples in São Paulo, Brazil. *Microb. Drug Resist.* 2016, 22, 321–327. [CrossRef]
62. Alekshun, M.N.; Levy, S.B. Molecular Mechanisms of Antibacterial Multidrug Resistance. *Cell* 2007, 128, 1037–1050. [CrossRef] [PubMed]
63. Brandt, C.; Braun, S.D.; Stein, C.; Slickers, P.; Ehrlich, R.; Pletz, M.W.; Makarewicz, O. In silico serine β-lactamases analysis reveals a huge potential resistome in environmental and pathogenic species. *Nature* 2017, 7, 1–13. [CrossRef] [PubMed]
64. Olsen, I. New promising β-lactamase inhibitors for clinical use. *Eur. J. Clin. Microbiol. Infect. Dis.* 2015, 34, 1303–1308. [CrossRef] [PubMed]
65. Lupof, A.; Papp-Wallace, K.M.; Sendi, P.; Bonomo, R.A.; Endimiani, A. Non-Phenotypic Tests to Detect and Characterize Antibiotic Resistance Mechanisms in Enterobacteriaceae. *Diagn. Microbiol. Infect. Dis.* 2013, 77, 179–194. [CrossRef] [PubMed]
66. Diene, S.M.; Rolain, J.-M. Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, *Pseudomonas* and *Acinetobacter* species. *Clin. Microbiol. Infect.* 2014, 20, 831–838. [CrossRef]
67. Poirel, L.; Wenger, A.; Bille, J.; Bernabeu, S.; Naas, T.; Nordmann, P. SME-2-Producing *Serratia marcescens* Isolate from Switzerland. *Antimicrob. Agents Chemother.* 2007, 51, 2282–2283. [CrossRef]
68. Queenan, A.M.; Bush, K. Carbapenemases: The Versatile -Lactamases. *Clin. Microbiol. Rev.* 2007, 20, 440–458. [CrossRef]
90. Meini, M.; Lıarrull, L.I.; Vila, A.J. Overcoming differences: The catalytic mechanism of metallo-\(\beta\)-lactamas. *FEBS Lett.* 2015, 589, 3419–3432. [CrossRef]

91. Mlynarcık, P.; Roderova, M.; Kolar, M. Primer Evaluation for PCR and its Application for Detection of Carbapenemases in Enterobacteriaceae. *Jundishapur J. Microbiol.* 2016, 9, 1–6. [CrossRef]

92. Pascual, A.; Pintadoc, V.; Rodríguez-Bañoa, J.; Miró, J.M. Carbapenemase-producing Enterobacteriaceae: The end of the antibiotic era? *Enferm. Infecce. y Microbiol. Clínica* 2014, 32, 1–3. [CrossRef]

93. Bonelli, R.R.; Moreira, B.M.; Picón, R.C. Antimicrobial resistance among Enterobacteriaceae in South America: History, current dissemination status and associated socioeconomic factors. *Drug Resist. Updates* 2014, 17, 24–36. [CrossRef]

94. Bratu, S.; Landman, D.; Haag, R.; Recco, R.; Eramo, A.; Alam, M.; Quale, J. Rapid spread of carbapenem resistant *Klebsiella pneumoniae* in New York City: A new threat to our antibiotic armamentarium. *Arch Intern. Med.* 2005, 165, 1430–1435. [CrossRef] [PubMed]

95. Bush, K. Proliferation and significance of clinically relevant \(\beta\)-lactamas. *Ann. N. Y. Acad. Sci.* 2013, 1277, 84–90. [CrossRef] [PubMed]

96. Bush, K.; Bradford, P.A. \(\beta\)-Lactams and \(\beta\)-Lactamase Inhibitors: An Overview. *Cold Spring Harb. Perspect. Med.* 2016, 6, 1–22. [CrossRef] [PubMed]

97. Bush, K.; Pucci, M.J. New antimicrobial agents on the horizon. *Biochem. Pharmacol.* 2011, 82, 1528–1539. [CrossRef]

98. Clark, J.D.; Catro, J.P.F.; Compton, C.; Lee, H.; Nunery, W. Orbital cellulitis and corneal ulcer due to *Cedecea*. First reported case and review of the literature. *Orbit* 2016, 35, 140–143. [CrossRef]

99. Cuzon, G.; Naas, T.; Correa, A.; Quinn, J.P.; Villegas, M.V.; Nordmann, P. Dissemination of the KPC-2 carbapenemase in non-*Klebsiella pneumoniae* enterobacterial isolates from Colombia. *Int. J. Antimicrob. Agents* 2013, 42, 59–62. [CrossRef]

100. Falcone, M.; Mezzatesta, M.L.; Perilli, M.; Forcella, C.; Giordano, A. Infections with VIM-1 metallo\(\beta\)-lactamase-producing *Enterobacter cloacae* and their correlation with clinical outcome. *J. Clin. Microbiol.* 2009, 47, 3514–3519. [CrossRef]

101. Gaynes, R.; Edwards, J.R. Overview of Nosocomial Infections Caused by Gram-Negative Bacilli. *Clin. Infect. Dis.* 2005, 41, 848–854.

102. Heggendorn, L.H.; Gomes, S.W.C.; Silva, N.A.; Vargas, R.G.; Póvoa, H.C.C. Epidemiological profile and antimicrobial susceptibility of microorganisms isolated from nosocomial infections. *Rev. Saúde e Meio Ambiente RESMA* 2016, 2, 26–47.

103. Jácome, P.R.L.A.; Alves, L.R.; Jácome-Júnior, A.T.; Silva, M.J.B.; Lima, J.L.C.; Araújo, P.S.R.; Lopes, A.C.S.; Maciel, M.A.V. Detection of bla SPM-1, bla KPC, bla TEM and bla CTX-M genes in isolates of *Pseudomonas aeruginosa, Acinetobacter spp.* and *Klebsiella spp.* from cancer patients with healthcare-associated infections. *J. Med. Microbiol.* 2016, 65, 658–665. [CrossRef]

104. Kordon, A.O.; Abdelhamed, H.; Ahmed, H.; Park, J.Y.; Karsi, A.; Pinchuk, L.M. Phagocytic and bactericidal properties of channel catfish peritoneal macrophages exposed to *Edwardsiella ictaluri* live attenuated vaccine and wild-type strains. *Front. Microbiol.* 2018, 8, 1–11. [CrossRef] [PubMed]

105. Lavagnoli, L.S.; Bassetti, B.R.; Kaiser, T.D.L.; Kutz, K.M.; Junior, C.C. Factors associated with acquisition of carbapenem-resistant Enterobacteriaceae. *Rev. Lat. Am. Enfermagem* 2017, 25, 1–7. [CrossRef] [PubMed]

106. Manageiro, V.; Ferreira, E.; Rodrigues, J.; Sampaio, D.A.; Vieira, L.; Pereira, P.; Rodrigues, P.; Palos, C.; Caniça, M. *NDM-1-Producing Providencia Stuartii Isolates in a Portuguese Hospital; Réunion Interciciplinaire de Chimiothérapie Anti-Infectieuse*: Paris, France, 2015; p. 35.

107. Pontes, D.S.; Araújo, R.S.A.; Dantas, N.; Scott, L.; Scott, M.T.; Moura, R.O.; Mendonça-Junios, F.J.B. Genetic Mechanisms of Antibiotic Resistance and the Role of Antibiotic Adjuvants. *Curr. Top. Med. Chem.* 2018, 18, 42–74. [CrossRef]

108. Assawatheptawee, K.; Tansawai, U.; Kiddee, A.; Thongngen, P.; Punyadi, P.; Romgaew, T.; Kongthai, P.; Sumpradit, T.; Niumsup, P.R. Occurrence of Extended-Spectrum and AmpC-Type \(\beta\)-Lactamase Genes in *Escherichia coli* isolated from Water Environments in Northern Thailand. *Microbes Environ.* 2017, 32, 293–296. [CrossRef]

109. Dixon, N.; Fowler, R.C.; Yoshizumi, A.; Horiyama, T.; Ishii, Y.; Harrison, L.; Geyer, C.N.; Moland, E.S.; Thomson, K.; Hanson, N.D. IMP-27: A Unique Metallo\(\beta\)-Lactamase Identified in Geographically Distinct Isolates of *Proteus mirabilis*. *Antimicrob. Agents Chemother.* 2016, 60, 6418–6421. [CrossRef] [PubMed]
89. Hussain, M.A.; Wang, W.; Sun, C.; Gu, L.; Liu, Z.; Yu, T.; Ahmad, Y.; Jiang, Z.; Hou, J. Molecular Characterization of Pathogenic Salmonella Spp From Raw Beef In Karachi, Pakistan. *Antibiotics* 2020, 9, 73, 1–16.

90. Song, W.; Lee, K.M.; Kim, H.S.; Kim, J.S.; Kim, J.; Jeons, S.H.; Roh, K.H. Clonal spread of both oxyimino-cephalosporin- and cefoxitin-resistant Klebsiella pneumoniae isolates co-producing SHV-2a and DHA-1-beta-lactamase at a burns intensive care unit. *Int. J. Antimicrob. Agents* 2006, 28, 520–524. [CrossRef]

91. Sugumar, M.; Kumar, K.M.; Manoharan, A.; Anbarasu, A.; Ramaiah, S. Detection of OXA-1 beta-Lactamase Gene of Klebsiella pneumoniae from Blood Stream Infections (BSI) by Conventional PCR and In-Silico Analysis to Understand the Mechanism of OXA Mediated Resistance. *PLoS ONE* 2014, 9, 1–8. [CrossRef]

92. Cubero, M.; Calatayud, L.; Tubau, F.; Ayats, J.; Peña, C.; Martin, R.; Liñares, J.; Domínguez, M.A.; Ardanuy, C. Clonal spread of Klebsiella pneumoniae producing OXA-1 betalactamase in a Spanish hospital. *Int. Microbiol.* 2013, 16, 227–233.

93. Mahalleh, R.G.D.; Dahmardeh, J.; Rad, N.S. The frequency of bla Verona imipenemase and bla imipenemase genes in clinical isolates of Pseudomonas aeruginosa in therapeutic centers of Zahedan. *Immunopathol. Persa* 2018, 4, e31. [CrossRef]

94. Shi, W.F.; Li, K.; Ji, Y.; Jiang, Q.B.; Wang, Y.Y.; Shi, M.; Mi, Z.H. Carbapenem and cefoxitin resistance of Klebsiella pneumoniae strains associated with porin OmpK36 loss and DHA-1 beta-lactamase production. *Braz. J. Microbiol.* 2013, 44, 435–442. [CrossRef] [PubMed]

95. Ramadan, A.A.; Abdelaziz, N.A.; Amin, M.A.; Aziz, R.K. Novel blaCTX-M variants and genotype-phenotype correlations among clinical isolates of extended spectrum beta lactamase-producing Escherichia coli. *Sci. Rep.* 2019, 9, 4224. [CrossRef] [PubMed]

96. Hajjej, Z.; Gharsallah, H.; Naija, H.; Boutiba, I.; Labbene, I.; Ferjani, M. Idcases. Successful treatment of a Carbapenem-resistant Klebsiella pneumoniae carrying bla(OXA-48), bla(VIM-2), bla(CMY-2) and bla(SHV-) with high dose combination of imipenem and amikacin. *Idcases* 2016, 4, 10–12. [CrossRef] [PubMed]

97. Pournajaf, A.; Rajabnia, R.; Razavi, S.; Solgi, S.; Ardebili, A.; Yaghoubi, S.; Khodabandeh, M.; Yahyapour, Y.; Emadi, B.; Irajian, G. Molecular characterization of carbapenem-resistant Acinetobacter baumannii isolated from pediatric burns patients in an Iranian hospital. *Trop. J. Pharm. Res.* 2018, 17, 135–141. [CrossRef]

98. Gajul, S.V.; Mohite, S.T.; Datkhile, K.D.; Kakade, S.V.; Mangalagi, S.S.; Wavare, S.M. Prevalence of Extended Spectrum Beta Lactamase Genotypes in Klebsiella pneumoniae from Respiratory Tract Infections at Tertiary Care Hospital. *J. Krishna Inst. Med. Sci. Univ.* 2019, 8, 66–75.

99. Chikwendu, C.I.; Ibe, S.N.; Okpokwasili, G.C. Detection of bla(SHV) and bla(TEM) beta-lactamase genes in multi-resistant Pseudomonas isolates from environmental sources. *Afr. J. Microbiol. Res.* 2011, 5, 2067–2074. [CrossRef]

100. Dhanji, H.; Patel, R.; Wall, R.; Doumith, M.; Patel, B.; Hope, R.; Livermore, D.M. Woodford N. Variation in the genetic environments of bla(CTX-M-15) in Escherichia coli from the faeces of travellers returning to the United Kingdom. *J. Antimicrob. Chemother.* 2011, 66, 1005–1012. [CrossRef]

101. Zhou, Y.; Zhu, X.H.; Hou, H.Y.; Lu, Y.F.; Yu, J.; Mao, L.; Mao, L.Y.; Sun, Z.Y. Characteristics of diarrheagenic Escherichia coli among children under 5 years of age with acute diarrhea: A hospital based study. *BMC Infect. Dis.* 2018, 18, 63. [CrossRef]

102. Chen, T.L.; Chang, W.C.; Kuo, S.C.; Lee, Y.T.; Chen, C.P.; Siu, L.K.; Cho, W.L.; Fung, C.P. Contribution of a Plasmid-Borne bla(OXA-58) Gene with Its Hybrid Promoter Provided by IS1006 and an ISAba3-Like Element to beta-Lactam Resistance in Acinetobacter Genomic Species 13TU. *Antimicrob. Agents Chemother.* 2010, 54, 3107–3112. [CrossRef]

103. Asgin, N.; Otlu, B.; Cakmakliogullari, E.K.; Celik, B. High prevalence of TEM, VIM, and OXA-2 beta-lactamases and clonal diversity among Acinetobacter baumannii isolates in Turkey. *J. Infect. Dev. Ctries.* 2019, 13, 794–801. [CrossRef]

104. Li, J.L.; Ji, X.L.; Deng, X.H.; Zhou, Y.F.; Ni, X.Q.; Liu, X.K. Detection of the SHV genotype polymorphism of the extended-spectrum beta-lactamase-producing Gram-negative bacterium. *Biomed. Rep.* 2015, 3, 261–265. [CrossRef] [PubMed]

105. Mathys, D.A.; Mathys, B.A.; Mollenkopf, D.F.; Daniels, J.B.; Wittum, T.E. Enterobacteriaceae Harboring AmpC (bla(CMY)) and ESBL (bla(CTX-M)) in Migrantry and Nonmigrantry Wild Songbird Populations on Ohio Dairies. *Vector-Borne Zoonotic Dis.* 2017, 17, 254–259. [CrossRef] [PubMed]
106. Khari, F.I.M.; Karunakaran, R.; Rosli, R.; Tay, S.T. Genotypic and Phenotypic Detection of AmpC beta-lactamases in Enterobacter spp. Isolated from a Teaching Hospital in Malaysia. PLoS ONE 2016, 11, e0150643.

107. Pruthvishree, B.S.; Kumar, O.R.V; Sivakumar, M.; Tamta, S.; Sunitha, R.; Sinha, D.K.; Singh, B.R. Molecular characterization of extensively drug resistant (XDR), extended spectrum beta-lactamases (ESBL) and New Delhi Metallo beta-lactamase-1 (blaNDM1) producing Escherichia coli isolated from a male dog-a case report. Vet. Arhiv 2018, 88, 139–148. [CrossRef]

108. Kim, Y.T.; Kim, T.U.; Baik, H.S. Characterization of extended spectrum beta-lactamase genotype TEM, SHV, and CTX-M producing Klebsiella pneumoniae isolated from clinical specimens in Korea. J. Microbiol. Biotechnol. 2006, 16, 889–895.

109. Ma, L.; Siu, L.K.; Lin, J.C.; Wu, T.L.; Fung, C.P.; Wang, J.T.; Lu, P.L.; Chuang, Y.C. Updated molecular epidemiology of carbapenem-non-susceptible Escherichia coli in Taiwan: First identification of KPC-2 or NDM-1-producing E-coli in Taiwan. BMC Infect. Dis. 2013, 13, 599. [CrossRef]

110. Yang, D.K.; Liang, H.J.; Gao, H.L.; Wang, X.W.; Wang, Y. Analysis of drug-resistant gene detection of blaOXA-like genes from Acinetobacter baumanii. Genet. Mol. Res. 2015, 14, 18999–19004. [CrossRef]

111. Azzab, M.M.; El-Sokkary, R.H.; Tawfeek, M.M.; Gebriel, M.G. Multidrug-resistant bacteria among patients with ventilator-associated pneumonia in an emergency intensive care unit, Egypt. East. Mediterr. Health J. 2016, 22, 894–903. [CrossRef]

112. Balkan, I.I.; Aygün, G.; Aydın, S.; Mutcalı, S.I.; Kara, Z.; Ku¸skucu, M.; Öztürk, R. Blood stream infections due to K. pneumoniae-lactamase Klebsiella pneumoniae carbapenemase 2-producing, ST11 international high-risk clone in Hungary, 2009–2013. J. Med. Microbiol. 2016, 65, 1020–1027. [CrossRef]

113. Kis, Z.; Toth, A.; Janvari, L.; Damjanova, I. Countrywide dissemination of a DHA-1-type plasmid-mediated AmpC beta-lactamase-producing Klebsiella pneumoniae ST11 international high-risk clone in Hungary, 2009–2013. J. Med. Microbiol. 2016, 65, 1020–1027. [CrossRef]

114. Gugliandolo, A.; Caio, C.; Mezzatesta, M.L.; Rifici, C.; Bramanti, P.; Stefani, S.; Mazzon, E. Successful ceftazidine-avibactam treatment of MDR-KPC-positive Klebsiella pneumoniae infection in a patient with traumatic brain injury A case report. Medicine 2017, 31, e7664. [CrossRef] [PubMed]

115. Farzana, R.; Shamsuzzaman, S.M.; Mamun, K.Z. Isolation and molecular characterization of New Delhi metallo-beta-lactamase-1 producing superbug in Bangladesh. J. Infect. Dev. Ctries. 2013, 7, 161–168. [CrossRef] [PubMed]

116. Souli, M.; Galani, I.; Antoniadou, A.; Papadomichelakis, E.; Poulakou, G. An outbreak of infection due to β-lactamase Klebsiella pneumoniae carbapenemase 2-producing K. pneumoniae in a Greek University Hospital: Molecular characterization, epidemiology, and outcomes. Clin. Infect. Dis. 2010, 50, 364–373. [CrossRef] [PubMed]

117. Sun, F.; Zhou, D.; Wang, Q.; Feng, J.; Feng, W.; Luo, W.; Zhang, D.; Liu, Y.; Qiu, X.; Yin, Z.; et al. The first report of detecting the blaSIM-2gene and determining the complete sequence of the SIM-encoding plasmid. Clin. Microbiol. Infect. 2016, 22, 347–351. [CrossRef] [PubMed]

118. Toombs-Ruane, L.J.; Benschop, J.; Priest, P.; Murdoch, D.R.; French, N.P. Multidrug resistant Enterobacteriaceae in New Zealand: A current perspective. N. Z. Veter. J. 2017, 65, 62–70. [CrossRef]

119. Wang, Y.; Jiang, X.; Xu, Z.; Ying, C.; Yu, W.; Xiao, Y. Identification of Raoultella terrigena as a Rare Causative Agent of Subungual Abscess Based on 16S rRNA and Housekeeping Gene Sequencing. Can. J. Infect. Dis. Med. Microbiol. 2016, 1, 1–4. [CrossRef] [PubMed]

120. Marques, J.B.; Bonez, P.C.; Agerott, V.A.; Flores, V.C.; Dalmolin, T.V.; Rossi, G.G.; Forno, N.L.F.D.; Bianchini, B.V.; Mizdal, C.R.; Siqueira, F.S.; et al. Molecular characterization of Enterobacteriaceae resistant to carbapenem antimicrobials. Rev. Bras. de Patol. Médica Lab. 2015, 51, 162–165. [CrossRef]

121. Kralik, P.; Ricchi, M. A basic guide to real time PCR in microbial diagnostics: Definitions, parameters, and everything. Front. Microbiol. 2017, 8, 1–9. [CrossRef]

122. Stefaniak, L.A.; Duarte, E.L.; Nishiyama, S.A.B.; Nakano, V. Resistência bacteriana: A importância das beta-lactamases. Rev. UNING 2005, 4, 123–137.

123. Álvarez, L.M.A.; García, J.M.G.; Hernández, M.D.P.; González, S.M.; Gutiérrez, J.J.P. Utility of Phenotypic and Genotypic Testing in the Study of Mycobacterium tuberculosis Resistance to First-Line Anti-Tuberculosis drugs. Arch. Broncopneumol. 2017, 53, 192–198. [CrossRef]
124. Filho, D.B.F.; Rocha, E.C.; Júnior, J.A.S.; Paranhos, R.; Neves, J.A.B.; Silva, M.B. Desvendando os Mistérios do Coeficiente de Correlação de Pearson: O retorno. Leviathan-Cad. Pesqui. Políti 2014, 8, 66–96.

125. Rodrigues, R.L.; Nascimento, H.F.; Menezes, G.L.; Lopes, A.R.; Nevoa, J.C.; Soares, W.C.S.; Santiago, S.B.; Barbosa, M.S. Contribuição ao estudo comparativo do diagnóstico laboratorial clássico e molecular de Helicobacter pylori: Uma abordagem investigativa. Rev. Acadêmica do Inst. de Ciências da Saúde 2016, 2, 18–25.

126. Jarlier, V.; Nicolas, M.H.; Fournier, G.; Philippon, A. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. Rev. Infect. Dis. 1998, 10, 867–878. [CrossRef] [PubMed]

127. Brazilian Committee on Antimicrobial Susceptibility Testing. Orientações do EUCAST para Detecção de Mecanismos de Resistência e Resistências Específicas de Importância Clínica e/ou Epidemiológica. 2015. EUCAST. Versão 1.0. Available online: http://brcast.org.br/documentos (accessed on 16 February 2018).

128. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing-Informational Supplement M100-S22; Versão 27; CLSI: Wayne, PA, USA, 2017.

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