Resistance Profile of *P. aeruginosa* isolates to β-lactams antimicrobials

Perfil de resistência da *P. aeruginosa* isola para β-lactams antimicrobianos

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ABSTRACT
β-lactamases are enzymes that hydrolyze the β-lactam ring, inactivating the action of β-lactam antibiotics. The objective of this work was to diagnose phenotypically and molecularly 14 β-lactamase resistance genes expressed in *Pseudomonas aeruginosa* and to correlate the results found. A total of 99 samples of *Pseudomonas aeruginosa* were selected and the antibiogram was performed. Real-time PCR is being performed using the Sybr Green system to amplify the genes corresponding to the resistances found in phenotyping. Of the 99 samples, 14 were identified as phenotypically resistant to the antimicrobial Aztreonam. The *bla*SME, *bla*OXA, *bla*GIM genes were simultaneously found in three (21.4%) samples. According to the statistical test, when evaluating the amplification results obtained for piperacillin + tazobactam, the molecular method was more sensitive for the detection of the gene coding for multidrug resistance, presenting values of ($p < 0.05$). This information suggests gene researches are more sensitive and specific when compared with to the antibiogram test.

Keywords: Antibiogram, Antimicrobial Resistance, β-lactams, Genes, Molecular Diagnosis.

RESUMO
As β-lactamases são enzimas que hidrolisam o anel β-lactama, inativando a ação dos antibióticos β-lactâmicos. O objetivo deste trabalho foi diagnosticar fenotipicamente e molecularmente 14 genes de resistência à β-lactamase expressos em Pseudomonas aeruginosa e correlacionar os resultados encontrados. Um total de 99 amostras de Pseudomonas aeruginosa foram selecionadas e o antibiograma foi realizado. A PCR em tempo real está sendo realizada utilizando o sistema Sybr Green para amplificar os genes correspondentes às resistências encontradas na fenotipagem. Das 99 amostras, 14 foram identificadas como fenotipicamente resistentes ao antimicrobiano Aztreonam. Os genes blaSME, blaOXA e blaGIM foram encontrados simultaneamente em três (21,4%) amostras. De acordo com o teste estatístico, ao avaliar os resultados da amplificação obtidos para piperacilina + tazobactam, o método molecular foi mais sensível para a detecção do gene que codifica para multirresistência, apresentando valores de ($p < 0,05$). Essas informações sugerem que as pesquisas genéticas são mais sensíveis e específicas quando comparadas ao teste de antibiograma.

Palavras-chave: Antibiograma, Resistência Antimicrobiana, β-lactâmicos, Genes, Diagnóstico Molecular.

1 INTRODUCTION

Pseudomonas aeruginosa is one of the most causative agent of infection associated with antimicrobial resistance and health care between isolated and genetic crescent information (1). Commonly used antipseudomonal drugs include penicillins with beta-lactamase inhibitors, certain cephalosporins, carbapenems, colistin, fluoroquinolones and aminoglycosides (2). The treatment of infections by *P. aeruginosa*, can become a challenge due to the capacity of this bacteria to be resistant to antibiotics by intrinsic and acquired mechanisms (3).

Antimicrobial resistance is one of the two main public health problems from the antimicrobial generation, including *P. aeruginosa* resistant multidrug (MDR). A prevalence of resistance is increasing in different parts of the world. *P. aeruginosa* frequently develops resistance to multiple classes of antibiotics and can to present XDR (extra-drug-resistant) and PDR (pan-drug-resistant) resistance (4).

Antibacterial resistances are routinely determined by classic phenotypic techniques. The disc diffusion and microdilution methods are considered to be the most important for detecting all the types of resistance mediated by carbapenems (5).

On the other hand, the technology used for PCR reactions (DNA Polymerase Reaction), represents a powerful technique in microbial diagnostics. In the area of bacterial diagnosis, it can substitute culture techniques, especially when there are necessary tests for fast and sensitive diagnosis. The accuracy is defined as the degree of

agreement measures in specific conditions, using statistical methods, such as standard deviation or confidence limit (6).

The most adequate antimicrobial treatments were established by the European Antimicrobial Susceptibility Committee (EUCAST) and or Clinical and Laboratory Standards Institute (CLSI) (7, 8). However, to evaluate the gene resistance that remains in the hospital it is crucial to determine the route of dissemination and content, that is the reason for more adequate methodologies (9).

P. aeruginosa species are between the bacteria with the potential to cause hospital infection; this specie is considered an important pathogen that causes respiratory infection. It is can easily find hospitalized patients, mainly using antimicrobials and invasive procedures (10).

This bacteria presents too multiple resistance to antimicrobials, in which it can happen naturally from the bacterial ability of adapting to different environmental conditions. The indiscriminate use of antimicrobials allows greater exposure to bacterial antimicrobial and opportunistic acquisition mechanisms of resistance. Antimicrobial resistance is a problem of public health at the world level (11).

One of the main mechanisms of resistance to antibiotics is the production of β-lactamases, bacterial enzymes that confer resistance to a wide variety of β-lactam antibiotics depending on the type of β-lactamase enzyme produced (12). The β-lactam belongs to the class of the antimicrobials that are more used clinically. These drugs are represented by penicillins, cephalosporins, monobactams and carbapenems (13).

The objectives of this study are to correlate the profile of resistance of *P. aeruginosa* using phenotypic and genotypic methodologies. The genes that encode resistance to β-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 has been hypothesized that molecular methods will improve accuracy, efficiency and acuity in comparison to the classical method of phenotyping. In addition, it can be released in short time; helping to improve the effectiveness of antibiotic therapy.

2 RESULTS

In this study 99 isolates of *P. aeruginosa* were analyzed, 90% originated from cannula of tracheostomized children, 5% of manual resuscitators and 5% were isolated from corneas. When evaluating antimicrobial resistance, 2/99 (2%) of the isolates were

resistant to all antimicrobials tested in this study (ATM, CAZ, IMP and PPT). The results showed that the highest antibiotic resistance was found in ATM (14.1%), the Figure 1 shows the presence of the antimicrobial resistance of *P. aeruginosa*.

The phenotypic profiles found in the antibiogram were analyzed to verify if the isolated ones of this study that presented resistance profiles showed multidrug resistance (MDR), the obtained data revealed positivity. According to the phenotype experiments, 14/99 (14.1%) of *P. aeruginosa* isolated were resistant to ATM; simultaneously, 6/14 (42.8%) of the same isolated resistant to antimicrobials ATM also presented resistance to CAZ, 3/14 (21.4%) presented resistance to IMP and 2/14 (14.8%) were resistant to PPT.

According to the resistance profile, bacteria can be classified as MDR, XDR and PDR. For this study it was considered that MDR were the samples that showed resistance to more than one antimicrobial agent, according to the classification published by Hidron et al., (18). The bacteria classified as XDR are epidemiologically resistant to multiple antimicrobials, but also considered the threatening profile of becoming resistant to all or almost all antimicrobial agents defined for a given classification (19).

Other data demonstrate that 99 isolated ones, 9/99 (9.1%) presented resistance to CAZ; simultaneously 4/9 (44.4%) the same isolated resistant to CAZ were also resistant to ATM, 3/9 (33.3%) resistant to IMP and 2/9 (22.2%) were resistant to PPT. In this research, the resistance to IMP was also evaluated and 8/99 (8.1%) of *P. aeruginosa* presented resistance; About 37.5% of isolated resistant to IMP were also resistant to ATM and CAZ and 2/8 (25%) were resistant to PPT.

Continuing the phenotype tests, of the 99 isolates, 4/99 (4.1%) were resistant to PPT; at the same time, 3/4 (75%) of the isolated had resistance to ATM, 2/4 (50%), had resistance to CAZ and 2/4 (50%) were resistant to IMP. The most frequent phenotype of resistance was presented by the antimicrobial ATM and denotes a concern for public health considering that bacterial genus have a certain facility for transmitting resistance information, as will be discussed later in Table 3.

Following the analyzes of the results of this work, resistance to Extended-spectrum beta-lactamases (ESBL), AmpC, Carbapenemics (CARBA) and MBL of the 99 isolated of *P. aeruginosa* were observed and 82 (82.8%) were found to be positive for the production of ampicillin - AmpC and 100% of the isolated presented negative results for the other tests, the data are in Table 2.

A literature review was carried out to determine the phenotypic resistance 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.

Table 3 was the result of a literature review of peer-reviewed articles that were selected on the PubMed platform over the past 15 years. The studies originated from different countries (Europe, Japan, Iran, Korea and Brazil) and characterized the possible genes encoding antimicrobial resistance used in *P. aeruginosa* (1; 20).

From the results obtained in the classical phenotype tests, qPCR experiments were performed using the Sybr Green variant for amplification of plasmid genes, which encode resistance to β-lactam antimicrobials. The results of the amplification showed that there was positivity for 12 (87.7%) of the 14 β-lactam genes analyzed in this study. Fisher's exact test (non-parametric test) was used to verify the statistical correlation and specificity of the tests applied in this study taking into account the results found in the antibiogram with those obtained in the qPCR reactions. The correlation of phenotypic and genotypic results is shown in Table 4.

In this study 14 from 99 samples were identified as phenotypically resistant to the ATM antimicrobial . Were done the amplification of the resistance gene, that second the literature, correspond to phenotyp tests and, the blaSME, blaOXA, blaGIM genes were simultaneously found in three (21.4%) samples. According to the statistical test, when evaluating the amplification results obtained for PPT, the molecular method was more sensitive for the detection of the gene coding for multidrug resistance, presenting values of ($p < 0.05$). This information suggests that gene research is more sensitive and specific compared to the antibiogram test.

Continuing the research, 9 isolated ones with phenotypic resistance to the antimicrobial CAZ were identified. Of these, 2/9 (22.2%) the genes *bla*GIM, *bla*SME, *bla*OXA, *bla*SIM, *bla*CTX-M and *bla*NDM were amplified simultaneously. Three antimicrobial resistant isolates ATM, IMP and CAZ simultaneously amplified the *bla*SME and *bla*OXA genes. Eight (12.5%) resistant samples of IMP were also analyzed when the β-lactam resistance genes were investigated. In one of the isolated, the *bla*SME, *bla*OXA and *bla*GIM genes were amplified. On the other hand, four isolated were found resistant to PPT and in one of them (25%), the genes *bla*SME, *bla*VIM, *bla*TEM and *bla*SPM were amplified.

Of the 99 samples from this study, 64% did not present phenotypic resistance to any of the 14 β-lactams tested. However, 2/64 (3.1%) of the samples amplified the genes

*bla*SME, *bla*CTX-M, *bla*CMY, *bla*SPM, *bla*NDM, *bla*DHA, *bla*TEM. This information characterizes the need to investigate gene expression to determine the sensitivity and efficiency of each of the two techniques involved in this research.

Seven 7/14 (50%) of the isolated resistant to ATM showed positivity for the production of AmpC; of these, 2/7 (28.5%) isolated harbored simultaneously the *bla*SME gene. One of the isolated presented the *bla*OXA, *bla*KPC and *bla*CTX-M genes separately and the other isolated *bla*VIM genes, *bla*TEM and *bla*SPM. Among the samples that showed positivity for AmpC production, 7/82 (8.5%) were resistant to ATM. Fifty-five percent (5/9) of CAZ-resistant isolated were positive for AmpC production. Of these, 1/5 (20%) of the isolated harbored the genes *bla*SME, *bla*OXA, *bla*SIM, *bla*CTX-M and *bla*NDM. From 99 samples of the study, 82 presented AmpC production, in this way, 5/82 (6.1%) are resistant simultaneously to CAZ and AmpC.

Three 3/8 (37.5%) of the isolated resistant to IMP produced AmpC. If we consider that the study started with 99 samples and that 82 of them were resistant to AmpC, it is evaluated that there is a correspondence of 3/82 (3.6%), positive samples for AmpC, however, no genes were found that confer resistance to β-lactams.

Two 2/4 (50%) of the PPT-resistant isolated produced AmpC; of these, 1/2 (50%), of the isolated amplified the genes *bla*SME, *bla*VIM, *bla*TEM and *bla*SPM. When considering all samples, 2/82 (2.4%) were simultaneously producing AmpC and resistant to PPT.

This study shows that all *P. aeruginosa* that produced β-lactamase AmpC positive were resistant to multiple drugs. The maximum sensitivity was 89.1%, observed for IMP followed by 51.5% for PPT.

A characterization was performed regarding the number, molecular weight and presence of multiple plasmids in the 35 isolated that presented resistance profile. According to the observations, plasmids >10 kb were found in 14/14 isolated resistant to ATM; in 8/9 resistant to CAZ; in 6/8 resistant to IMP; in 3/4 PPT resistant. In this study, it was clarified that most of the isolated contained plasmids >10 Kb. Multiple plasmids were also found in isolated resistant to ATM (4/14), CAZ (2/9) and IMP (1/8). Thus, if one considers the molecular weights and the presence of multiple plasmids with different profiles, such isolated are more likely to contain multiple resistance genes, which may justify the presence of MDR in some samples.

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 perfil must be the sequencing experiments that will be done in another study.

3 DISCUSSION

The results showed 14.1% ATM antibiotic resistance, the data obtained in this study is in according with those found in a retrospective study, which included 192 patients diagnosed with infections caused by *P. aeruginosa* in Romania from January 1, 2006 to December 31, 2012. The results demonstrated a resistance of 16.1% for ATM, 17.86% for PPT and for CAZ resistance was 26.2% (21).

In this study, the most frequent phenotype of resistance was presented by the antimicrobial ATM, however in one study conducted in India showed that all *P. aeruginosa* that produced β-lactamase were resistant to multiple drugs. The maximum resistance (48.5%) was observed for PPT and a smaller activity, when compared to the present study, was determined for IMP (10.9%) (22).

About 82% of the samples analyzed in this study were found to be positive for the production of ampicillin - AmpC and 100% of the isolated presented negative results for the other tests, but in a study among the patients who presented *Pseudomonas* spp., 100% were positive for AmpC production (23). The data of the authors collaborate to support that *P. aeruginosa* isolated have acquired the gene with capacity in the production of the ampicillinases.

The results found here suggested that gene research is more sensitive and specific compared to the antibiogram test. Gasparetto et al., (24), conducted a study in three university hospitals in Porto Alegre, RS, Brazil, and found the *bla*VIM and *bla*IMP genes in two of them. For the *bla*VIM gene, the isolated from hospital A presented 67% of positive positivity in the samples of *P. aeruginosa* and in hospital B 35.71% of positivity. The *bla*IMP gene presented 8.33% positivity in the isolates of hospital A and 21.43% of positivity in the isolated of hospital B (25). It is observed that there was a divergence of percentages found in the isolates of the study carried out in Porto Alegre - RS, Brazil, when compared to the isolated of this study. This difference can be justified by the heterogeneous distribution of resistance genes according to regionalization.

Brazilian authors have reported that the gene coding for the enzyme SPM-1, first described by Tolleman et al., (26), was the most prevalent in *P. aeruginosa* resistant to carbapenems and phenotypically producing MBL. The gene was found in percentages higher than 50% of the isolated, considering the different geographic regions (27).

However, the *bla*VIM and *bla*IMP genes, which encode resistance to MBLs, have also been identified in *P. aeruginosa* isolated (27; 28; 29).

In the study by Zavascki et al., (30), it was demonstrated that the presence of MBL-producing *P. aeruginosa* increased the risk of inadequate antimicrobial therapy, with a consequent increasing mortality of the patients. This aspect was not evaluated in the present study, but a relatively high percentage (9/12, 75%) of MBL-encoding genes was detected among phenotypically positive isolated.

In 2005, a cohort study, conducted at Hospital das Clínicas, UFU (Uberlândia Federal University) / Minas Gerais, Brazil, revealed a significant change in the gene epidemiology of *P. aeruginosa* isolated, in which only the *bla*SPM-1 gene (31). From 2011, the dissemination of MBL-producing isolated with plasmids containing the *bla*VIM gene (10) has been reported.

It was observed that several genes from this study were simultaneously amplified in samples that exhibited phenotypic resistance to the tested antimicrobials. Supporting this research and suggesting that resistance genes can spread to different continents, there a study using 892 samples of *P. aeruginosa* isolated has collected among January 2009 and December 2012 at Mackay Memorial Hospital - Taiwan has identified that 90/892 of *P. aeruginosa* isolated were screened for the carbapenem genes; 21/90 (23.33%), were positive for ESBL genes, and the following genes were identified: *bla*TEM, *bla*SHV, *bla*CTX, *bla*OXA (32). Another study compounds of 123 *P. aeruginosa* strains collected from the patient lower respiratory tract of 45, evaluated that 25 were samples were susceptible to both CAZ and IPM, 9 only to IPM and 36 only to CAZ; 53 strains were resistant to both drugs (33).

The results of this study revealed the importance of and the need to analyze phenotypic resistance as well as to determine antimicrobial susceptibility by beta-lactamase coding genes. The PCR technique allows the identification of highly zoonotic pathogens, directly from tissues, making the diagnosis faster and safer for laboratory workers (34; 35). As well as making it easier and more reliable to diagnose diseases caused by fastidious microorganisms (36).

A characterization was performed regarding the number, molecular weight and presence of multiple plasmids in isolated that presented resistance profile. According to Huang et al., (37), the presence of MDR is usually located in plasmids that have transferability to several resistance genes. This quote collaborates with the present study,

when we consider that there are samples of *P. aeruginosa*, isolated from different clinical specimens, in which similar plasmid profiles were found.

This resistance study for *P. aeruginosa* showed that of the 99 samples involved in the research, 14/99 (14.1%) of *P. aeruginosa* isolated were resistant to ATM; 9 (9.1%) presented resistance to CAZ; 8/99 (8.1%) of *P. aeruginosa* presented resistance to IMP; 4/99 (4.1%) were resistant to PPT.

The highest antibiotic sensitivity was found for PPT (95.9%) and the lowest resistance was found for IMP (8.1%). About 2/99 (2%) of the isolated were resistant to all tested antimicrobials.

When evaluating the profile of multiple drug resistance of *P. aeruginosa*, it was detected that in 9 isolated MDR were found. From those tested, 3 (21.4%) for ATM, 2 (22.2%) for CAZ, 2 (25%) for IMP and 2 (50%) for PPT. The percentage of MDR was 9/99 (9.1%) isolated, among them 5/9, represented by trachyostomized children and 4/9 isolated from MRI.

In this study, the percentage of ESBL-producing bacteria was 0% since, the test was negative for all isolated; 82 (82.8%) isolated showed positivity for AmpC production and 100% of the isolated presented negative results for the other tests. The percentages of genes found in the isolated were: *bla*SME in 100% of samples, *bla*OXA (50%), *bla*GIM (16.6%), *bla*KPC (16.6%), *bla*CTX- 6%), *bla*NDM (33.2%), *bla*VIM (16.6%), *bla*TEM (33.2%), *bla*SPM (33.2%), *bla*CMY (16.6%) and *bla*DHA).

When evaluating the genes encoding ATM resistance to isolated that simultaneously produce AmpC, it was found that in 7/14 resistant isolated, two harbored the *bla*SME gene. In 5/9 CAZ resistant isolated and AmpC producers, in one isolated, harbored the genes *bla*SME, *bla*OXA, *bla*SIM, *bla*CTX-M and *bla*NDM.

When analyzing 3/8 isolated resistant to IMP and producers of AmpC, none genes encoding resistance to β-lactams were found. When considering two isolated resistant to PPT and AmpC producers, only one of them amplified for the genes *bla*SME, *bla*VIM, *bla*TEM and *bla*SPM.

Relevant to report that 7/12 genes amplified in qPCR (*bla*SME, *bla*TEM, *bla*SPM, *bla*DHA, *bla*NDM, *bla*CMY and *bla*CTX-M) had not been detected in phenotyping, denoting the specificity of the molecular method compared to the classical phenotypic methodology.

4 MATERIALS AND METHODS

4.1 PHENOTYPIC ANALYZES

A total of 99 bacterial samples of *P. aeruginosa* species were stored in a bio-repository at the Laboratory. Bacteria come from the cannula of tracheostomized children (5 samples), mucosa of tonsils (89 samples), human corneas (5 samples), as well as respiratory equipment from a hospital service, Manual Resuscitators - MRI (5 samples). The isolates of human corneas originated from the Service of Verification of Deaths (SVO) of 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 biorrepository, these *P. aeruginosa* 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 aztreonam (ATM) 30 μg, ceftazidime (CAZ) 30 μg, imipenem (IMP) 10 μg and piperacillin-tazobactam (PPT) 100/10 μg. As a quality control, strains *P. aeruginosa* ATCC® 27853 and *Escherichia coli* ATCC® 35218 were used for combinations of β-lactam inhibitors / β-lactamases (8).

For the phenotypic detection of ESBL production, the *P. aeruginosa* was isolated and submitted to the approach disk technique modified using the methodology described by Jarlier et al., (14).

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 (15; 16).

Imipenem and meropenem (MEM) discs were used for the phenotypic investigation of carbapenemases and the interpretation of the sensitivity following the criteria established by CLSI (8). At least one bacteria that showed resistance of the carbapenems were submitted to the metallo-beta-lactamase (MBL) screening test, using the enzyme blockade method and following the recommendations of Brazilian National Public Health Agency (ANVISA) (17). The test used IMP (10μg) and MEM (10μg) disc, positioned parallel to two other imipenem and meropenem discs added with 10μL of EDTA.

For *P. aeruginosa*, in addition to the EDTA test, the modified Hodge test (MHT) was also performed. MHT consists of inoculating an *E. coli* ATCC 35218®. 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 (8).

4.2 MOLECULAR ANALYZES

For each bacteria, 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 LOW ROX - 100 reactions x 25 µL) 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 *Eco*R 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.

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Figure 1. Percentage of resistance and sensitivity of the *Pseudomonas aeruginosa* samples, against the antimicrobials tested.

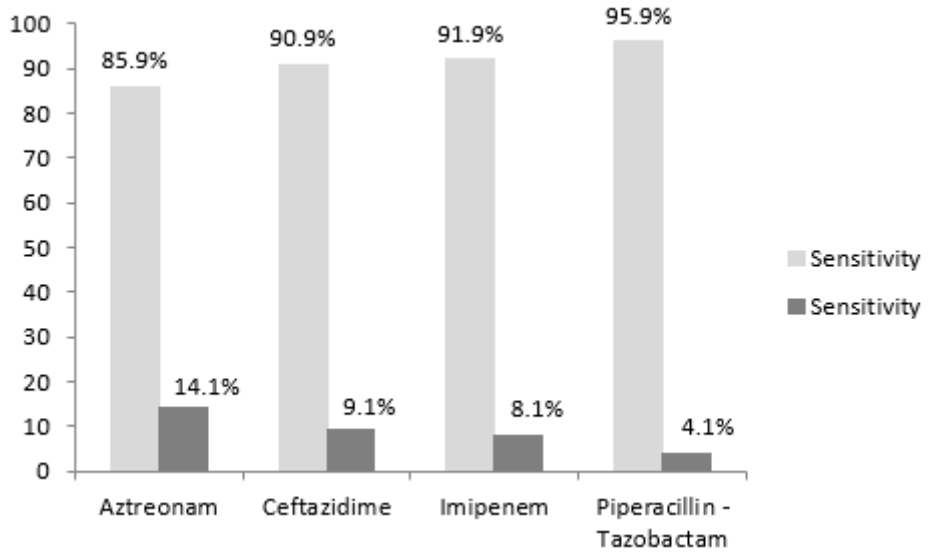

Table 1. Oligonucleotides used for amplification of the β-lactam resistance genes of this study.

Genes	Gene sequence from 5 'to 3'	Temperature of ringing	Quantity of bases	Access at the GenBank	Amplified fragment size
bla OXA	Forward: GGCAGCGGGTTCCCTTGTC	49.7	19	FN396876.1	171pb
	Reverse: CGATAATGGGCTGCAGCGG	49.7	19		
bla IMP	Forward: CCAGCGTACGGCCCACAGA	49.6	19	NG035455.1	138pb
	Reverse: GGTGATGGCTGTTGCGGCA	50.3	19		
bla NDM	Forward: CGGCCGCGTGCTGGTG	49.8	16	JN711113.1	182pb
	Reverse: GGCATAAGTCGCAATCCCCG	50.2	20		
bla SME	Forward: GGCGGCTGCTGTTTTAGAGAGG	50.9	25	KJ188748.1	184pb
	Reverse: GCAGCAGAAGCCATATCACCTAAT	50.3	22		
bla DHA	Forward: GCGGGCGAATTGCTGCAT	49.8	18	NG041043.1	183pb
	Reverse: TGGGTGCCGGGGTAGCG	50.1	17		
bla CMY	Forward: GGATTAGGCTGGGAGATGCTGAA	50.1	23	NG041279.1	158pb
	Reverse: CCAGTGGAGCCCGTTTTATGC	49.6	21		
bla TEM	Forward: TCCGTGTCGCCCTTATTCCC	49.6	20	KJ923009	165pb
	Reverse: CCTTGAGAGTTTTCGCCCCG	49.6	20		
bla SHV	Forward: GGCAGCGGGTTCCCTTGTC	49.7	19	FN396876.1	171pb
	Reverse: CGATAATGGGCTGCAGCGG	49.7	19		
bla VIM	Forward : GTTATGCCGCACCCACCCC	50.3	19	NG036099.1	194 pb
	Reverse: ACCAAACACCATCGGCAATCTG	49.7	22		
bla SPM	Forward: CGAAAATGCTTGATGGGACCG	50.3	21	DQ145284.1	147pb
	Reverse: CACCCGTGCCGTCCAAATG	49.7	19		
bla CTX	Forward: CTGAGCTTAGCGCGGCCG	50.1	18	FJ815279.1	189pb
	Reverse: AATGGCGGTGTTTAACGTCGG	50.0	21		
bla GIM	Forward: CGGTGGTAACGGCGCAGTG	50.2	19	JX566711.1	149pb
	Reverse: TGCCCTGCTGCGTAACATCG	50.2	20		

Tabela 2. Bibliographical survey concerning the phenotypic resistance of β-lactamases against the corresponding resistance genes.

Antimicrobials	*bla*$_{OXA}$	*bla*$_{IMP}$	*bla*$_{NDM}$	*bla*$_{SME}$	*bla*$_{DHA}$	*bla*$_{CMY}$	*Bla*$_{TEM}$	*bla* $_{SHV}$	*bla* $_{VIM}$	*bla* $_{KPC}$	*bla*$_{GIM}$	*bla*$_{SPM}$	*bla*$_{CTX-M}$	*bla*$_{SIM}$
Aztreonan	+	-	+	-	-	-	+	+	+	+	-	-	+	-
Ceftazidime	+	+	+	-	-	-	+	+	+	+	-	+	+	-
Imipenem	+	+	+	+	-	-	-	-	+	+	-	-	-	+
Piperacillin + Tazobactam	-	-	-	-	-	+	+	+	+	+	-	-	+	-

Legend: The positive sign (+) implies in the previous description of the literature of the gene in question, as well as the relation with the respective antimicrobial, while the negative sign (-) indicates that such relation was not found in the literature.

Table 3. Relative data on the resistance to ESBLs, tested in this study for *P. aeruginosa*.

Samples	ESBL	AmpC	CARBA	MBL	Samples	ESBL	AmpC	CARBA	MBL	Sampless	ESBL	AmpC	CARBA	MBL
1	-	+	-	-	34	-	+	-	-	67	-	+	-	-
2	-	+	-	-	35	-	+	-	-	68	-	+	-	-
3	-	+	-	-	36	-	+	-	-	69	-	+	-	-
4	-	+	-	-	37	-	+	-	-	70	-	+	-	-
5	-	+	-	-	38	-	+	-	-	71	-	+	-	-
6	-	+	-	-	39	-	-	-	-	72	-	+	-	-
7	-	+	-	-	40	-	-	-	-	73	-	+	-	-
8	-	+	-	-	41	-	+	-	-	74	-	+	-	-
9	-	+	-	-	42	-	+	-	-	75	-	+	-	-
10	-	+	-	-	43	-	-	-	-	76	-	-	-	-
11	-	+	-	-	44	-	+	-	-	77	-	+	-	-
12	-	+	--	-	45	-	-	-	-	78	-	+	-	-
13	-	+	-	-	46	-	+	-	-	79	-	+	-	-
14	-	+	-	-	47	-	+	-	-	80	-	+	-	-
15	-	+	-	-	48	-	+	-	-	81	-	+	-	-
16	-	-	-	-	49	-	+	-	-	82	-	-	-	-
17	-	+	-	-	50	-	+	-	-	83	-	-	-	-
18	-	+	-	-	51	-	-	-	-	84	-	+	-	-
19	-	+	-	-	52	-	-	-	-	85	-	+	-	-
20	-	-	-	-	53	-	+	-	-	86	-	+	-	-
21	-	+	-	-	54	-	-	-	-	87	-	+	-	-
22	-	+	-	-	55	-	-	-	-	88	-	+	-	-
23	-	+	-	-	56	-	+	-	-	89	-	+	-	-
24	-	+	-	-	57	-	+	-	-	90	-	+	-	-
25	-	+	-	-	58	-	+	-	-	91	-	+	-	-
26	-	+	-	-	59	-	+	-	-	92	-	+	-	-
27	-	+	-	-	60	-	+	-	-	93	-	+	-	-
28	-	+	-	-	61	-	+	-	-	94	-	+	-	-
29	-	+	-	-	62	-	-	-	-	95	-	-	-	-
30	-	+	-	-	63	-	+	-	-	96	-	-	-	-
31	-	+	-	-	64	-	+	-	-	97	-	+	-	-
32	-	+	-	-	65	-	+	-	-	98	-	-	-	-
33	-	+	-	-	66	-	+	-	-	99	-	+	-	-

Extended Spectrum β-lactamases (ESβL); Metalo-β-lactamases (MβL); Ampicillin (AmpC). Carbapenemase (CARBA).

Table 4. Correlation between phenotypic and genotypic resistance of 99 *P. aeruginosa* isolates

Antimicrobial	Isolated resistant (N/99)	Isolated that amplify	Exact Test by Fisher P	Genes β-lactams	Multidrug resistant	Exact Test by Fisher P	Plasmid Number	Plasmid profile
ATM	14 (14.1%)	3/14 (21.4%)	0.69	blaSME bla OXA blaGIM blaKPC blaCTX-M blaVIM blaTEM blaSPM	3/14 (21.4%)	0.69	4 4 14	1.5 Kb 2.5 Kb >10Kb
CAZ	9 (9.1%)	2/9 (22.2%)	0.23	blaGIM BlaSME blaOXA blaCTX-M blaSIM blaDNM	2/9 (22.2%)	0.23	2 2 8	1.5 Kb 2.5 Kb >10Kb
IMP	8 (8.1%)	1/8 (12.5%)	0.98	blaSME blaOXA blaGIM	2/8 (25%)	0.16	1 1 6	1.5 Kb 2.5 Kb >10Kb
PPT	4 (4.1%)	1/4 (25%)	0.18	blaSME blaVIM blaTEM blaSPM	2/4 (50%)	**0.02**	3	>10Kb