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Short Communication

Incidence and dissemination of genes encoding Aminoglycoside-Modifying Enzymes (AME's) in clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Recife, Brazil.

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Abstract: The aim of this study was to investigate the presence of three AME-encoding genes and to determine the resistance profiles of clinical *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates. To evaluate the susceptibility profile towards gentamicin and amikacin, the automated Vitek 2 Compact method (bioMérieux, Marcy-l' Etoile, France) was used. The presence of the aac(6')-Ib-cr, ant(3'')-Ia, and aph(3'')-Ia genes was analyzed in 35 *A. baumannii* and 63 *P. aeruginosa* isolates using PCR. Our investigation revealed diverse resistance profiles across the hospitals studied. Isolates of *A. baumannii* from Hospital 2 and *P. aeruginosa* from both hospitals exhibited significant resistance to the tested aminoglycosides, while *A. baumannii* isolates from Hospital 1 showed remarkable sensitivity. The aac(6')-lb-cr gene was detected in 55% of the isolates, ant-3''-la in 41%, and aph(3'')-la in 42%. This study reports the first occurrence of the aph(3'')-la gene in these two pathogens in Brazil. It was possible to correlate the phenotypic profile of anti-microbial sus-ceptibility with the genotypic profile of most isolates. These findings underscore the incidence and spread of these genes, highlighting the need for surveillance to implement measures to control hospital outbreaks.

Keywords: aminoglycosides; microbial dissemination; hospital environment; AMEs genes.

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1. Introduction

Among the causative agents of Healthcare-Associated Infections (HAIs), *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are classified as pathogens of critical priority by the World Health Organization [1]. These pathogens are ubiquitous in nature and have reduced susceptibility to adverse environmental conditions, which contributes to

their potential for transmissibility in the hospital environment [2]. The dynamics of HAIs caused by these pathogens have become a challenge because these isolates acquire mechanisms of resistance to various antimicrobial agents, making it difficult to choose an effective drug therapy and, consequently, increasing their spread and persistence in the hospital environment [2].

For many years, aminoglycoside antimicrobials have been the first choice for the treatment of bacterial infections; however, due to the emergence of new drugs, they have been disused. Currently, aminoglycosides have been increasingly used [3], which has led to a higher number of bacterial isolates becoming resistant to this class of antibiotics, thereby limiting therapeutic options [3, 4]. One of the main mechanisms of resistance to aminoglycosides is the production of Aminoglycoside-Modifying Enzymes (AMEs) that chemically modify the drug, reducing its affinity for ribosomes [4].

The aim of this study was to investigate the presence of three AME-encoding genes in clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* collected from two public hospitals in Recife, Pernambuco, Brazil. The study also aimed to compare the genetic data with the isolates' resistance profile to amikacin and gentamicin. The investigated genes were aminoglycoside acetyltransferases (aac (6')-Ib-cr), aminoglycoside phosphotransferases (aph (3'')-Ia) and aminoglycoside nucleotidyltransferases (an (3'')-Ia). This study provides insights into the dynamics of gene incidence and transmission in these pathogens.

2. Materials and Methods

The isolates obtained from two public hospitals in Recife from April to October 2016, were previously identified [2], of which 98 were molecularly characterized (35 *A. baumannii* and 63 *P. aeruginosa*). To evaluate the susceptibility profile towards gentamicin and amikacin, the automated Vitek 2 Compact method (bioMérieux, Marcy-l' Etoile, France) was used. This study was approved by the Research Ethics Committee of the Federal University of Pernambuco, Brazil.

Bacterial were grown in Brain Heart Infusion (BHI) broth for 18–24 h, and total DNA was extracted using the PureLink kit (Invitrogen) according to the manufacturer's protocol. The *aac (6')-Ib-cr*, *an (3'')-Ia*, and *aph (3'')-Ia* were detected using Polymerase Chain Reaction (PCR) with specific primer pairs and annealing temperatures [4, 5]. Amplicons were electrophoresed using 1.2% agarose gel and evaluated using a 100 bp DNA ladder marker (Invitrogen, Carlsbad, CA, USA).

3. Results

The results of this study provide valuable insights into the antibiotic resistance patterns of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates in two public hospitals in Recife, Brazil. Our findings demonstrate that the resistance profiles of these pathogens vary between hospitals, highlighting the importance of local surveillance to inform antibiotic prescribing practices and infection control measures.

In hospital 1, the majority of *A. baumannii* isolates were sensitive to both amikacin and gentamicin, with only one isolate showing intermediate resistance to amikacin. In contrast, in hospital 2, a high proportion of *A. baumannii* isolates were resistant to both amikacin and gentamicin, which suggests the presence of ongoing transmission of these resistant strains.

Interestingly, our study also identified different resistance profiles in *P. aeruginosa* isolates from both hospitals. In hospital 1, a higher proportion of isolates were resistant to gentamicin, while in hospital 2, a higher proportion were resistant to amikacin. These findings suggest that the resistance patterns of *P. aeruginosa* may also vary between hospitals and underscore the need for continued monitoring to better understand and control the spread of antibiotic resistance in these pathogens.

While our study provides important insights into the dynamics of antibiotic resistance in *A. baumannii* and *P. aeruginosa* isolates, there are several limitations that must be considered. For instance, the sample size was relatively small, and the study was limited to two public hospitals in a specific geographic location. Further research is needed to confirm our findings in larger and more diverse populations.

Despite these limitations, our study has important implications for clinical practice and policy. Our findings suggest that targeted surveillance programs and infection control measures are essential to combat the spread of antibiotic-resistant pathogens in hospitals. Additionally, promoting the rational use of antibiotics and implementing antibiotic stewardship programs may help to mitigate the development and spread of antibiotic resistance in the community.

Table 1. Susceptibility profile to aminoglycosides and genotype profile.

Isolates	**Susceptibility to aminoglycosides**		**AMEs genes**
	Amikacin	**Gentamycin**	
H1A. 438.4	S	S	*aac(6')-Ib-cr*
H1A.473.4	S	S	-
H1A. 557.4	S	S	-
H1A.749.4	R	S	-
H1A.789.4	S	S	ant(3'')-Ia
H1A.895.4	I	S	*aac(6')-Ib-cr*
H1A.898.4	**S**	**S**	aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia)
H1A.619.5	S	S	-
H1A.620.5	S	S	-
H1A.62.6	S	R	*aac(6')-Ib-cr*
H1A.223.6	S	S	-
H1A.452.6	S	S	-
H2A.8687	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H2A.8710-1	**R**	**R**	aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia
H2A.8747	R	R	-
H2A.8896	R	R	*aac(6')-Ib-cr*
H2A.9450	R	R	-

H2A.9733-2	R	R	*aac(6')-Ib-cr*
H2A.10865	R	R	*aac(6')-Ib-cr*
H2A.10986	R	R	aac(6')-Ib-cr, ant(3'')-Ia
H2A.11229	R	R	-
H2A.11471	R	R	-
H2A.11481	R	R	ant(3'')-Ia
H2A.11562	S	I	-
H2A.11580	R	R	-
H2A.11596	R	R	-
H2A.11531	R	R	*aac(6')-Ib-cr*
H2A.12156	R	R	*aac(6')-Ib-cr*
H2A.12166	R	R	-
H2A.14700	R	R	aph(3'')-Ia
H2A.14731	R	R	-
H2A.14808	S	R	*aac(6')-Ib-cr*
H2A.14900	I	R	-
H2A.14967	R	R	-
H2A.15166	R	R	-
H1P.535.4	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.538.4	R	R	*aac(6')-Ib-cr, aph(3'')-Ia*
H1P.554.4	**S**	**S**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.555.4	S	S	*ant(3'')-Ia, aph(3'')-Ia*
H1P.586.4	**S**	**S**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.661.4	**S**	**S**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.670.4	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H1P.680.4	R	R	*aac(6')-Ib-cr*
H1P.683.4	S	S	*ant(3'')-Ia, aph(3'')-Ia*
H1P.732.4	S	S	*aac(6')-Ib-cr*

H1P.810.4	R	R	-
H1P.824.4	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H1P.837.4	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H1P.876.4	R	R	aph(3'')-Ia
H1P.921.4	**S**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.528.5	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H1P.552.5	R	R	-
H1P.603.5	**S**	**S**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.666.5	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.717.5	R	R	*aac(6')-Ib-cr*
H1P.841.5	**S**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.936.5	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.1058.5	R	R	aph(3'')-Ia
H1P.1087.5	**S**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.1098.5	S	R	aph(3'')-Ia
H1P.64.6	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.75.6	**I**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.96.6	R	R	-
H1P.98.6	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.170.6	R	R	-
H1P.198.6	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.199.6	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H1P.235.6	R	R	*aac(6')-Ib-cr*
H1P.244.6	S	R	*ant(3'')-Ia, aph(3'')-Ia*
H1P.332.6	**S**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.432.6	**S**	**S**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.484.6	**S**	**S**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H1P.545.6	S	S	-

H2P.8305	S	R	*aac(6')-Ib-cr*
H2P.8410	I	S	-
H2P.8662	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H2P.8697	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H2P.8843	S	R	*ant(3'')-Ia*
H2P.8999	S	R	aph(3")-Ia
H2P.9128	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H2P.9362	R	R	*ant(3'')-Ia, aph(3'')-Ia*
H2P.9392	S	S	-
H2P.9367	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H2P.9474	S	S	*ant(3'')-Ia, aph(3'')-Ia*
H2P.9798	**S**	**I**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H2P.11256	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H2P.11394	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H2P.11557	R	R	*ant(3'')-Ia*
H2P.11739	I	S	*ant(3'')-Ia, aph(3'')-Ia*
H2P.11807	R	R	*ant(3'')-Ia*
H2P.11809	**R**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*
H2P.11877	S	R	*aac(6')-Ib-cr*
H2P.11914	R	R	*aac(6')-Ib-cr* *ant(3'')-Ia*
H2P.14325	R	R	**-**
H2P.14357	R	R	-
H2P.14685	R	R	*aac(6')-Ib-cr*
H2P.16304	R	R	*ant(3'')-Ia*
H2P.16025	**S**	**R**	*aac(6')-Ib-cr, ant(3'')-Ia, aph(3'')-Ia*

*H1A, *A. baumannii* - Hospital 1; H2A, *A. baumannii* - Hospital 2; H1P, *P. aeruginosa* - Hospital 1; H2P, *P. aeruginosa* - Hospital 2; - Absence of the 3 genes.

The study found that aac (6')-lb-cr was the most frequent gene observed, with 34% and 67% prevalence in *A. baumannii* and *P. aeruginosa* isolates, respectively. The ant-3''-la gene was found in 17% and 63% of *A. baumannii* and *P. aeruginosa* isolates, respectively, and aph (3'')-la was detected in 11% and 59% of *A. baumannii* and *P. aeruginosa* isolates, respectively. Interestingly, this study is the first to report the incidence of the aph (3'')-la gene in these two pathogens in Brazil.

The study also identified the co-occurrence of the three genes in 26 isolates. The prevalence of the genes in both hospitals was also evaluated, with aac (6')-lb-cr being the most prevalent gene in both hospitals, followed by ant-3''-la and aph (3'')-la. Table 1 presents an overview of the susceptibility profiles of the isolates against the tested aminoglycosides and their relationships with the AMEs genes.

Overall, the results demonstrate the importance of gene analysis for understanding the resistance/susceptibility profiles of bacterial isolates and their dynamics of incidence and spread in bacterial pathogens. It is also important to note that while *P. aeruginosa* had a greater positivity for the genes, this may be due to a more representative sample universe. However, the study cannot affirmatively conclude this, as in some situations, the number of bacteria may be lower, but they may contain the most significant arsenal gene, as previously evidenced by Silva et al. [6].

In summary, the study sheds light on the prevalence of aminoglycoside resistance genes in *A. baumannii* and *P. aeruginosa* isolates in Brazil, highlighting the importance of gene analysis for understanding bacterial resistance/susceptibility profiles and their dynamics.

4. Discussion

Infections caused by Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) microorganisms are extremely difficult to treat, and aminoglycosides are one of the main choices for treatment. However, with the increasing occurrence of resistance to this class of drugs, therapeutic options are limited [2, 6]. This scenario is concerning and demonstrates the need to monitor these pathogens in hospitals. The present study performed a comparative analysis between the susceptibility profiles of *A. baumannii* and *P. aeruginosa* isolates from two public hospitals in Recife and their genetic profile in relation to AMEs.

Different resistance profiles were identified in the investigated hospitals. *A. baumannii* isolates from hospital 2 and *P. aeruginosa* from both hospitals showed prominent resistance to the tested aminoglycosides, whereas *A. baumannii* isolates from hospital 1 showed remarkable sensitivity. A previous study reported amikacin as a treatment option for these microorganisms. However, studies have shown an increased amikacin resistance in both species [2, 3, 6] which was also verified in the present study.

The distinct resistance profile may be related to both pathogens and therapeutic practices used in each hospital. This difference demonstrates the importance of identifying and monitoring the microbiota and profile of each hospital, even when located in the same city. Previous study, such as those described by Lima et al. [2] have already identified these differences and highlighted the importance of continuous monitoring in these environments.

Considering the main mechanisms of resistance to these antimicrobials, AMEs have contributed to therapeutic failures, and aminoglycoside-resistant microorganisms generally possess more than one gene of this family, which are found in mobile elements, plasmids, and transposons [3,4]. The Aminoglycoside Acetyltransferase (AAC) group is the most prevalent and clinically relevant group, as it has many variants distributed worldwide. The *aac (6'')-Ib* is the most common in *A. baumannii* and *P. aeruginosa*, conferring resistance to aminoglycosides, such as amikacin, as well as to antimicrobials from other classes, such as tobramycin. Although genes encoding aminoglycoside phosphotransferases (APH) are less frequent in the non-glucose-fermenting bacilli group, *aph*

(3')-Ia, *aph (3')-IIb*, and *aph (3')-SAW* are among those mainly reported in these microorganisms. The aminoglycoside nucleotidyltransferase (ANT) group is less frequent in these species, with the most common genes include *ant (2')-Ia* and a*nt (3')-Ia* [3, 4].

The co-occurrence of the three genes was identified in 26 isolates (genotype VII), of which 2 were *A. baumannii* and 24 were *P. aeruginosa. P. aeruginosa* isolates showed a greater resistance to the tested antimicrobials. However, some isolates that possessed the three genes were not resistant to aminoglycosides. Therefore, the presence of these genes is not always sufficient to guarantee resistance, since it can cause genetic suppression, leading to gene silencing or lack of promoters in the region where the genes are inserted [7]. In contrast, some isolates that did not harbor the three genes were resistant to the tested aminoglycosides. In such cases, resistance may be related to the presence or association of other resistance and virulence mechanisms, such as biofilm formation. Lima et al. [2] reported that these isolates, which did not present the investigated genes, have a moderate to strong capacity to form biofilms, which may contribute to resistance to these antimicrobials.

Biofilm forms a barrier that prevents the penetration and action of antimicrobials at their site of action. Another important characteristic of biofilms is the lack of oxygen and low metabolism of the bacterial cells in the biofilm, preventing aminoglycosides from being actively absorbed in the cytoplasmic membrane and reach the ribosome [7].

Araújo et al. [8] analyzed 243 *P. aeruginosa* isolates, of which only 5% harbored *aac (6')-Ib-cr*. In the present study, 34% of *A. baumannii* and 67% of *P. aeruginosa* isolates harbored this gene, demonstrating a constant increase in genetic dissemination in Brazil. Both species showed higher positivity for *aac (6')-Ib-cr*, corroborating with other studies that reported a higher prevalence of this gene [9, 10]. Other studies have recently reported the presence of this gene in other gram-negative bacteria (*Klebsiella pneumoniae* and *Providencia stuartii*) in the city of Recife [6].

The *ant(3'')-Ia* is less abundant in *A. baumannii* and *P. aeruginosa* and is less frequently reported in Brazil [3, 9]. However, the present study showed that 17% of *A. baumannii* isolates harbored this gene, which demonstrates an increase in its occurrence and reveals the need for monitoring in these environments. Similarly, this gene was present in 63% of *P. aeruginosa* isolates investigated in the present study, revealing an increase in its incidence when compared to that reported in previous studies [11].

In addition, *aph (3'')-Ia* was detected in 11% of *A. baumannii* isolates and 59% of *P. aeruginosa* isolates. The variants *aph (3')- Ia* and *aph (3')-Ib* are more common in both pathogens and have a high incidence in other regions, especially in Asian countries and some North American countries [11, 12]. The *aph (3'')-Ia* variant is rarely detected in these species [13, 14]. The present study is the first to detect this variant in *A. baumannii* and *P. aeruginosa* isolates in Brazil, thus demonstrating its increased occurrence and worldwide dissemination.

The worldwide spread of pathogens and consequently their resistance genes has emerged at an accelerated pace in recent years, making pathogens highly adaptive to various environments. The increase in international mobility and socioeconomic patterns facilitates this transmission, directly contributing to the geographic trends in microbial resistance compared to previous years. Efforts are needed to curb the inappropriate use of antimicrobials worldwide, along with greater vigilance to understand the role of the movement of humans, animals, and food products in the transmission of resistance [15].

5. Conclusions

The current study provides important insights into the prevalence and dissemination of aminoglycoside resistance genes (aac (6')-lb-cr, ant-3''-la, and aph (3'')-la) in *A. baumannii* and *P. aeruginosa* clinical isolates. The results are concerning, as they highlight the lack of effective surveillance and monitoring protocols for these pathogens, which can lead to increased hospitalization and mortality rates. The findings emphasize the urgent need for monitoring the antimicrobial resistance patterns of these pathogens to reduce

their impact on public health. By implementing effective measures to control outbreaks in the hospital environment, we can contribute to the reduction of morbidity and mortality rates caused by these microorganisms. Therefore, these results underscore the importance of continuous monitoring of AME-encoding genes in clinical isolates to ensure the timely and appropriate management of these infections.

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