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Emergence of polymyxin B-resistant *Acinetobacter baumannii* in hospitals in Rio de Janeiro

Emergência de Acinetobacter baumannii *resistente a polimixina B em hospitais do Rio de Janeiro*

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ABSTRACT

Introduction: *Acinetobacter baumannii* has been considered a prevalent pathogen in hospitals, raising concern in the medical community due to its broad spectrum of antimicrobial resistance. Since it is a subject that arouses much interest, it has been increasingly studied. Due to the emergence of multidrug-resistant (MDR) Gram-negative bacteria, the use of polymyxins was reestablished. The polymyxins have been considered the only option for the treatment of severe infections caused by MDR *A. baumannii*. **Objective:** To investigate the susceptibility profile of *A. baumannii* to polymyxin B. **Material and method:** 92 clinical isolates from two public hospitals in the Rio de Janeiro city were studied using broth microdilution method. **Results:** Most of the isolates were resistant to polymyxin B, 81.5% (n = 75), and minimum inhibitory concentration (MIC) values ranged between 4-64 mcg/ml. **Conclusion:** These results are a concern since currently the polymyxins have been considered the most effective therapeutic option against MDR isolates of *A. baumannii*.

Key words: multidrug-resistance; *Acinetobacter baumannii*; polymyxin B; hospital infection.

INTRODUCTION

Acinetobacter baumannii has been considered a prevalent pathogen in hospitals, raising concerns for the medical community due to its broad-spectrum for antimicrobial resistance[1]. It is an opportunistic pathogen, with a high incidence in immunocompromised individuals, particularly those who have had prolonged hospitalization.

The World Health Organization (WHO) identified the antimicrobial resistance as one of the three major human health problems[2]. In this context, *A. baumannii* is among the most common and dangerous multidrug-resistant (MDR) pathogens[3, 4], because of that, this subject has been increasingly studied[5].

Due to high levels of MDR Gram-negative bacteria, the use of polymyxins was reestablished, especially in cases of carbapenem-resistant isolates. The polymyxins are cyclic peptides positively charged that interact with the lipid A component of the lipopolysaccharides (LPS) present in the outer membrane of Gram-negative bacteria[6]. On the other hand, this class of antimicrobials has serious adverse effects, which are mainly nephrotoxicity (mainly acute renal failure) and neurotoxicity. Other effects are also described, such as, for example, allergies, fever and eosinophilia[7]. In general, resistance to polymyxins is still uncommon among non-fermenting Gram-negative microorganisms, although reports of *A. baumannii* – resistance to these agents have increasingly been described[8, 9]. Currently, polymyxins have been considered the only option for the treatment of severe infections caused by MDR *A. baumannii* [10-13].

OBJECTIVE

This study aimed to investigate the susceptibility profile of 92 clinical isolates of *Acinetobacter baumannii* from two public hospitals of the Rio de Janeiro city between 2010 and 2011.

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MATERIAL AND METHOD

Bacterial identification

All isolates were previously identified in hospitals studied by automated VITEK 2 system (bioMérieux Inc., Hazcwood, Mo.), using cards for identification of Gram-negative (GN, reference 21341). In the laboratory, the isolates were tested for their purity and their morphotinctorial characteristics were observed by Gram staining technique. The isolates were also subjected to the following biochemical tests: motility, citrate utilization test (Simmons), indole production, oxidation-fermentation glucose in Hugh-Leifson medium, cytochrome oxidase production and growth at 42ºC, according to Murray *et al*. (2010)[14]. The confirmation of identification was performed by amplifying and sequencing of the *rpoB* gene, which encodes the β subunit of ribonucleic acid (RNA) polymerase, considering an identity level of 99%-100%[15]. Isolates were stored in Tryptone Soy Broth (TSB) (Difco®), containing 20% glycerol (v/v) and kept in the freezer at -20ºC and -70ºC.

Determination of the minimum inhibitory concentration (MIC) for polymyxin B by broth microdilution method

The isolates were evaluated by the broth microdilution method using the protocol and the breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI)[16]. The following antimicrobial concentrations were chosen for testing: 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.25 µg/ml and 0.125 µg/ml. After isolates growth at 37ºC for 24 hours in Nutrient Agar medium, bacterial suspensions were carried out in sterile saline 0.85% compatible to 0.5 McFarland standard (1.8×10^8 CFU/ml), which were subsequently diluted in the range of 1:100 in cation-adjusted Mueller Hinton broth (CAMH) medium pH 7.3, to obtain a final cell concentration of 1.8×10^6 CFU/ml. Using enzyme-linked immunosorbent assay (ELISA) microplate plate, 50 µl of polymyxin B were added into the wells at different concentrations followed by 50 µl of bacterial suspensions. Bacterial inoculation in the wells was performed in the range of up to 30 minutes, and the plates were homogenized on the bench in rotational movement and incubated for 24 hours at 37ºC. The reading was performed by visual inspection, in which the first concentration which showed no bacterial growth (medium turbidity) was assumed as the MIC. Some wells of the plate were used as sterility controls of the antimicrobial and the bacterial growth; the reference strain *Escherichia coli* ATCC 25922 was used as test control.

RESULTS

Bacterial identification

Conventional biochemical tests were performed for all isolates from the two public hospitals in Rio de Janeiro city. These tests were important at the first stage of confirmation of identification. To confirm the identification of these isolates to the species-level, it was necessary to perform a molecular identification by polymerase chain reaction (PCR) amplification and sequencing of *rpoB* gene. This methodology enables the species-level identification of isolates, and all belonged to *Acinetobacter baumannii* specie (similarity 99%-100%). As a result, all 92 isolates that were previously subjected to biochemical and genotypic tests were identified as *Acinetobacter baumannii*.

Distribution of isolates

Among the 92 *A. baumannii* isolates studied, most were from the hospital 1 (81.5%; $n = 75$). The remaining isolates originated from patients from hospital 2.

The *A. baumannii* isolates were obtained from different specimen types. Among those collected at hospital 1, the majority were from urine (22.7%; $n = 17$), followed by blood and catheter (18.7%; $n = 14$, each) and tracheal aspirates (16%; $n = 12$), fewer in number were originated from wound and bronchoalveolar lavage. The origin of the specimen was not identified in 1.3% (n=1) of the isolates collected in this hospital (**Figure 1**). About the hospital 2, the clinical specimen was not identified in 35.3% (n=6) of the studied isolates (**Figure 2**).

Determination of MIC

Polymyxin MIC of the isolates was determined using the microdilution broth method[16]. By this method, we observed a small number of susceptible isolates to this antimicrobial (18.5%; $n = 17$). Among the susceptible isolates, two presented MIC of 1 µg/ml and the other 15 presented MIC of 2 µg/ml. Most of the isolates were polymyxin B-resistant (81.5%; $n = 75$), showing MIC values between 4-64 µg/ml (**Figure 3**).

About the susceptible isolates to polymyxin B ($n = 17$), only four were present in hospital 2. Among the polymyxin B-resistant isolates ($n = 75$), it could be noted that most of them were collected from urine (18.8%; $n = 15$).

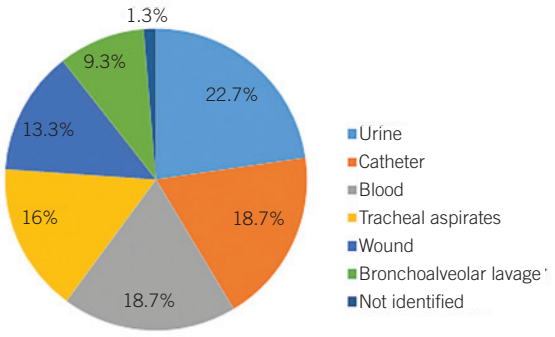

FIGURE 1 – *Percentage of 75* Acinetobacter baumannii *isolates from hospital 1 according to the source of specimens*

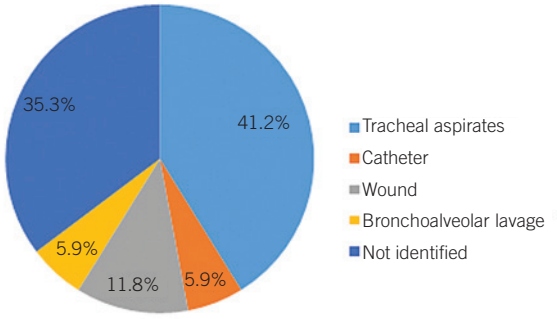

FIGURE 2 – *Percentage of 17* Acinetobacter baumannii *isolates from hospital 2 according to the source of specimens*

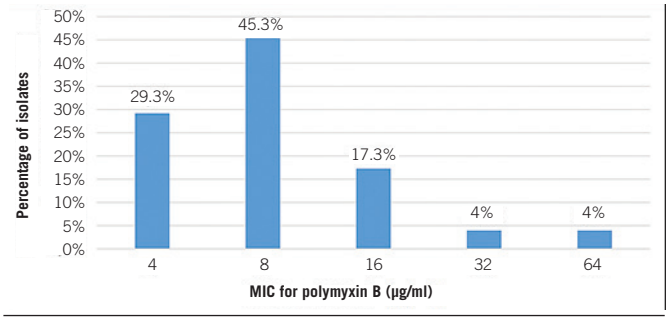

FIGURE 3 – *Distribution of MIC in relation to polymyxin B-resistant isolates*

MIC: minimum inhibitory concentration.

DISCUSSION

Acinetobacter species are commonly associated with hospital-acquired infections or healthcare-associated infections (HAI), especially in developing countries[17, 18]. The *A. baumannii* specie has become a major problem in the nineties and, over the years, this organism has become an important HAI-causing agent in the world[19]. In this regard, the study of *A. baumannii* isolates collected in hospitals is of great importance, since this microorganism is associated with several outbreaks in Brazil and worldwide[20, 21].

The species-level identification of isolates among *Acinetobacter* species is often problematic. Currently, *Acinetobacter* species are identified by molecular techniques[22, 23] such as sequencing the gene encoding the subunit 16S RNA ribosomal (*16S rRNA*), which is one of the most common method used for bacterial identification[24]. However, the main limitation is because this gene is so preserved, which does not enables, in case of *Acinetobacter*, differentiating all species[25]. Other genes that encode proteins such as *rpoB* gene, which have been used to identify isolates of *Acinetobacter spp*., allow to differentiate most species of this microorganism[26].

In our study, we observed a large number of isolates of *A. baumannii* polymyxin B-resistant, which differs from some studies in Iran and Mexico, that show isolates with 100% of susceptibility to polymyxin B[26]. In some studies, polymyxin B was the only antimicrobial that showed efficacy against *A. baumanni*[27-29]. Moreover, in isolates collected in Latin America in 2001, this antimicrobial showed excellent activity, since 96,4% of the isolates were susceptible to it. In this study, only six isolates were classified as polymyxin-resistant, they were from three different Brazilian hospitals; only one of them was also carbapenem-resistant[27].

Despite these reports on high susceptibility of isolates to polymyxins, in the past few years the intensive use of polymyxins has led to the selection of *A. baumannii* isolates resistant to these antibiotics; the resistance rates of 40.7% in Spain and 30.6% in Korea were already reported[8, 9]. More recently, isolates of *A. baumannii* polymyxin B-resistant were also recovered in Iran and the United States[30, 31]. Some studies have predicted the increased resistance to polymyxin, as observed in our work. Rolain *et al*. (2011)[32] reported that resistance rates would increase once the use of antimicrobial become more common, for example, in the treatment of infection caused by *A. baumannii* carbapenem-resistant, leading to the development of resistance by adjustment to selective pressure exerted by the antimicrobial[32, 33].

It is evident that the rational use of antibiotics is essential to prevent outbreaks of MDR *A. baumannii* infections, since the emergence of this specie is usually associated with selective pressure of prolonged use of broad-spectrum antimicrobials[34]. Carbapenems are still among the drugs of choice for the treatment of infections caused by *A. baumannii*, however increasing number of resistance reports of this microorganism

to these antibiotics has become a great concern for the medical community[35, 36]. As an alternative to the use of carbapenems, polymyxins B and E have been used as the most effective therapy for treatment of serious infections caused by MDR *A. baumannii*, even with reports of isolates of this pathogen resistant to these antimicrobials[37].

CONCLUSION

In this study, by evaluating the MIC for polymyxin B, we observed high percentages of resistance among the studied isolates (81.5%; $n = 75$), which increasingly limits the therapeutic options available for the treatment of infections caused by *A. baumannii*. Therefore, strict infection control measures to prevent the emergence and spread of such isolates should be adopted.

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RESUMO

Introdução: Acinetobacter baumannii *tem sido considerado um patógeno prevalente nos hospitais, gerando preocupação na comunidade médica por conta de seu extenso espectro de resistência aos antimicrobianos. Por ser um assunto que desperta muito interesse, tem sido cada vez mais estudado. Devido à emergência de bactérias Gram-negativas resistentes a múltiplas drogas (MDR), o uso de polimixinas foi reestabelecido. As polimixinas têm sido consideradas a única opção para o tratamento de infecções graves causadas por* A. baumannii *MDR.* ***Objetivo:*** *Investigar o perfil de suscetibilidade de* A. baumannii *à polimixina B.* ***Material e método:*** *Foram estudados 92 isolados clínicos provenientes de dois hospitais da rede pública do município do Rio de Janeiro por meio da técnica de microdiluição em caldo.* ***Resultados:*** *A maioria dos isolados foi resistente à polimixina B, 81,5% (*n = *75), apresentando valores de concentração inibitória mínima (CIM) entre 4-64 mcg/ml.* ***Conclusão:*** *Esses resultados são preocupantes, já que atualmente as polimixinas têm sido consideradas a opção terapêutica mais eficaz contra isolados de* A. baumannii *MDR.*

Unitermos: resistência microbiana a medicamentos; Acinetobacter baumannii*; polimixina B; infecção hospitalar.*

REFERENCES

1. Cerqueira GM, Peleg AY. Insight into Acinetobacter baumannii pathogenicity. IUBMB Life. 2011; 63(12): 1055-60. PubMed PMID: 21989983.

2. Bassetti M, Ginocchio F, Mikulska M. New treatment options against gram-negative organisms. Crit Care. 2011; 15(2): 215. PubMed PMCID: PMC3219411.

3. Rice LB. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J Infect Dis. 2008; 197(8): 1079-81. PubMed PMID: 18419525.

4. Gbaguidi-Haore H, Dumartin C, L'Hériteau F, et al. Antibiotics involved in the occurrence of antibiotic-resistant bacteria: a nationwide multilevel study suggests differences within antibiotic classes. J Antimicrob Chemother. 2013; 68(2): 461-70. PubMed PMID: 23075690.

5. Husni RN, Goldstein LS, Arroliga AC, et al. Risk factors for an outbreak of multi-drug-resistant Acinetobacter nosocomial pneumonia among intubated patients. Chest. 1999; 115: 1378-92. PubMed PMID: 10334156.

6. Hancock RE, Chapple DS. Peptide antibiotics. Antimicrob Agents Chemother. 1999; 43(6): 1317-23. PubMed PMCID: PMC89271.

7. Mendes CA, Burdmann EA. Polymyxins – review with emphasis on nephrotoxicity. Rev Assoc Med Bras. 2009; 55(6): 752-9. PubMed PMID: 20191233.

8. Ko KS, Suh JY, Kwon KT, et al. High rates of resistance to colistin and polymyxin B in subgroups of Acinetobacter baumannii isolates from Korea. J Antimicrob Chemother. 2007; 60(5): 1163-7. PubMed PMID: 17761499.

9. Arroyo LA, Mateos I, González V, Aznar J. In vitro activities of tigecycline, minocycline, and colistin-tigecycline combination against multi- and pandrug-resistant clinical isolates of Acinetobacter baumannii group. Antimicrob Agents Chemother. 2009; 53(3): 1295-6. PubMed PMID: 19075049.

10. Young ML, Bains M, Bell A, Hancock RE. Role of Pseudomonas aeruginosa outer membrane protein OprH in polymyxin and gentamicin resistance: isolation of an OprH-deficient mutant by gene replacement techniques. Antimicrob Agents Chemother. 1992; 36(11): 2566-8. PubMed PMID: 1336952.

11. Kapoor K, Jajoo M, Dublish S, Dabas V, Gupta S, Manchanda V. Intravenous colistin as therapy for nosocomial infections caused by multidrugresistant Pseudomonas aeruginosa and Acinetobacter baumannii. Clin Infect Dis. 1999; 28(5): 1008-1. PubMed PMID: 10452626.

12. Appleman MD, Belzberg H, Citron DM, et al. In vitro activities of nontraditional antimicrobials against multiresistant Acinetobacter baumannii strains isolated in an intensive care unit outbreak. Antimicrob Agents Chemother. 2000; 44(4): 1035-40. PubMed PMID: 10722508.

13. Pogue JM, Cohen DA, Marchaim D. Editorial commentary: polymyxin-resistant Acinetobacter baumannii: urgent action needed. Clin Infect Dis. 2015; 60(9): 1304-7. PubMed PMID: 25632011.

14. Murray PR, Rosenthal KS, Pfaller MA. Microbiologia médica. 6 ed. Rio de Janeiro: Elsevier; 2010.

15. Gundi VA, Dijkshoorn L, Burignat S, Raoult D, La Scola B. Validation of partial rpoB gene sequence analysis for the identification of clinically important and emerging Acinetobacter species. Microbiol. 2009; 155 (Pt7): 2333-41. PubMed PMID: 19389786.

16. Clinical and Laboratory Standands Institute (CLSI). Performance standards for antimicrobial susceptibility testing. CLSI approved standard M100-S25. Wayne, Pennsylvania: CLSI; 2015.

17. Baumgart AM, Molinari MA, Silveira AC. Prevalence of carbapenem resistant Pseudomonas aeruginosa and Acinetobacter baumannii in high complexity hospital. Braz J Infect Dis. 2010; 14(5): 433-6. PubMed PMID: 21221469.

18. Cieslinski JM, Arend L, Tuon FF, et al. Molecular epidemiology characterization of OXA-23 carbapenemase-producing Acinetobacter baumannii isolated from Brazilian hospitals using repetitive sequence-based PCR. Diagn Microbiol Infect Dis. 2013; 77(5): 337-40. PubMed PMID: 24074766.

19. Munoz-Price LS, Weinstein RA. Acinetobacter infection. N Engl J Med. 2008; 358(12): 1271-82. PubMed PMID: 18354105.

20. Valentine SC, Contreras D, Tan S, Real LJ, Chu S, Xu HH. Phenotypic and molecular characterization of Acinetobacter baumannii clinical isolates from nosocomial outbreaks in Los Angeles County, California. J Clin Microbiol. 2008; 46(8): 2499-2507. PubMed PMID: 18524965.

21. Valenzuela JK, Thomas L, Partridge SR, van der Reijden T, Dijkshoorn L, Iredell J. Horizontal gene transfer in a polyclonal outbreak of carbapenem resistant Acinetobacter baumannii. J Clin Microbiol. 2007; 45(2): 453-60. PubMed PMID: 17108068.

22. Karah N, Haldorsen B, Hegstad K, Simonsen GS, Sundsfjord A, Samuelsen Ø. Species identification and molecular characterization of Acinetobacter spp. blood culture isolates from Norway. J Antimicrob Chemother. 2011; 66(4): 738-44. PubMed PMID: 21393175.

23. Ahmed SS, Alp E. Genotyping methods for monitoring the epidemic evolution of A. baumannii strains. J Infect Dev Ctries. 2015; 9(4): 347-54. PubMed PMID: 25881522.

24. Custovic A, Smajlovic J, Tihic N, Hadzic S, Ahmetagic S, Hadzagic H. Epidemiological monitoring of nosocomial infections caused by Acinetobacter baumannii. Med Arch. 2014; 68(6): 402-6. PubMed PMID: 25648217.

25. Alvarez-Buylla A, Culebras E, Picazo JJ. Identification of Acinetobacter species: is Bruker biotyper MALDI-TOF mass spectrometry a good alternative to molecular techniques? Infect Genet Evol. 2012; 12(2): 345-920. PubMed PMID: 22266021.

26. Najar Peerayeh S, Karmostaji A. Molecular identification of resistance determinants, integrons and genetic relatedness of extensively drug resistant Acinetobacter baumannii isolated from hospitals in Tehran, Iran. Jundishapur J Microbiol. 2015; 8(7): e27021. PubMed PMID: 26421140.

27. Tognim MC, Gales AC, Penteado AP, Silbert S, Sader HS. Dissemination of IMP-1 metallo-beta-lactamase-producing Acinetobacter species in a Brazilian teaching hospital. Infect Control Hosp Epidemiol. 2006; 27(7): 742-7. PubMed PMID: 16807851.

28. Viana GF, Santos Saalfeld SM, Garcia LB, Cardoso CL, Pelisson M, Tognim MC. Evolution of antimicrobial resistance of Acinetobacter baumannii in a university hospital. Lett Appl Microbio. 2011; 53(3): 374-8. PubMed PMID: 21707678.

29. Liu Q, Li W, Feng Y, Tao C. Efficacy and safety of polymyxins for the treatment of Acinectobacter baumannii infection: a systematic review and meta-analysis. PLoS One. 2014; 9(6): e98091. PubMed PMID: 24911658.

30. Bahador A, Taheri M, Pourakbari B, et al. Emergence of rifampicin, tigecycline, and colistin-resistant Acinetobacter baumannii in Iran; spreading of MDR strains of novel international clonevariants. Microb Drug Resist. 2013; 19(5): 397-406. PubMed PMID: 23768166.

31. Lesho E, Yoon EJ, McGann P, et al. Emergence of colistin-resistance in extremely drug-resistant Acinetobacter baumannii containing a novel pmrCAB operon during colistin therapy of wound infections. J Infect Dis. 2013; 208(7): 1142-51. PubMed PMID: 23812239.

32. Rolain JM, Roch A, Castanier M, Papazian L, Raoult D. Acinetobacter baumannii resistant to colistin with impaired virulence: a case report from France. J Infect Dis. 2011; 204(7): 1146-7. PubMed PMID: 21881132.

33. Barin J. Hetero- and adaptive resistance to polymyxin B in OXA-23-producing carbapenem-resistant Acinetobacter baumannii isolates. 2013; 12(15): 15. PubMed PMID: 23819554.

34. Mak JK, Kim MJ, Pham J, Tapsall J, White PA. Antibiotic resistance determinants in nosocomial strains of multidrug-resistant Acinetobacter baumannii. J Antimicrob Chemother. 2009; 63(1): 47-54. PubMed PMID:18988680.

35. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo RA. Global challenge of multidrug-resistant Acinetobacter baumannii. Antimicrob Agents Chemother. 2007; 51(10): 3471-84. PubMed PMID: 17646423.

36. Nicasio AM, Kuti JL, Nicolau DP. The current state of multidrug-resistant gram negative bacilli in North America. Pharmacotherapy. 2008; 28(2): 235-49. PubMed PMID: 18225969.

37. Pogue JM, Cohen DA, Marchaim D. Editorial commentary: polymyxin-resistant Acinetobacter baumannii: urgent action needed. Clin Infect Dis. 2015; 60(9): 1304-7. PubMed PMID: 25632011.

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