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# *Acinetobacter baumannii*: disseminação e evolução das beta-lactamases em três hospitais do norte do Paraná durante 20 vinte anos

Maringá
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Dissertação apresentada ao Programa de Pós-Graduação em Biociências e Fisiopatologia do Departamento de Análises Clínicas e Biomedicina, Centro de Ciências da Saúde da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Mestre em Biociências e Fisiopatologia.
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Orientadora: Prof.ª Dr.ª Maria Cristina Bronharo Tognim

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*“Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”*

*“A persistência é o menor caminho do êxito.”*

***Charles Chaplin***

*Acinetobacter baumannii*: disseminação e evolução das beta-lactamases em três hospitais do norte do Paraná durante 20 vinte anos


**RESUMO**

A resistência aos antimicrobianos, especialmente em *Acinetobacter baumannii* (Ab) tornou-se um sério problema de saúde pública mundial e a produção de β-lactamases, constituem o principal mecanismo envolvido. Assim, o estudo teve como objetivo avaliar a evolução das β-lactamases e a susceptibilidade aos β-lactâmicos em amostras de Ab isoladas ao longo dos últimos 20 anos. Isolados de Ab (1994 a 2014) foram recuperados de distintos pacientes de unidades terapia intensivas de três hospitais da região norte do Paraná. Para avaliar a similaridade entre as amostras foi utilizado técnica de *Enterobacterial Repetitive Intergenic Consensus* - ERIC-PCR. Genes de carbapenemases (*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-58}}$ , *bla*$_{\text{OXA-143}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{NDM}}$ e *bla*$_{\text{KPC}}$) e β-lactamases de espectro estendido-ESBL (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{PER}}$, *bla*$_{\text{GES}}$ e *bla*$_{\text{VEB}}$) foram detectados por PCR-multiplex. Testes de susceptibilidade a diferentes grupos de agentes antimicrobianos foram determinadas por ágar diluição. Entre as 176 amostras encontradas, 84,6% (149/176) apresentaram resistência aos carbapenêmicos. Quando analisado por períodos, a taxa de resistência a imipenem e meropenem passou de 0% no período de 1994 a 1996 para 66,7% em 2004, e 100% em 2014. A redução da susceptibilidade foi acompanhada com a evolução da presença do gene *bla*$_{\text{OXA-23}}$, que foi de 0% em 1994 para 98,4% em 2014, já o gene *bla*$_{\text{OXA-51}}$ foi identificado em 100% dos isolados. De maneira inédita no Brasil, reportamos a presença do gene *bla*$_{\text{CTX-M-15}}$ (Genbank KT945131), em quatro isolados de Ab carreando gene *bla*$_{\text{OXA-23,}}$ sendo pertencentes a dois clones, no qual um deles foi encontrado em dois hospitais em épocas diferentes (2009 e 2014). A sensibilidade a polimixina B foi de 100% e a cefalosporinas em média 10%. Os demais genes pesquisados (carbapenemase e ESBL) não foram detectados em nenhum isolado. A genotipagem demonstrou a presença de 92 *clusters* dentre eles, 11 foram detectados por mais de um período em diferentes hospitais. Os resultados demonstram um aumento de isolados carreando *bla*$_{\text{OXA-23}}$ nos últimos vinte anos, consequentemente aumentando a resistência aos carbapenêmicos. Esses dados e a detecção de CTX-M-15 reforçam a necessidade de implantação de medidas de vigilância para controle da disseminação de Ab resistentes.



**Palavras - chave:** *Acinetobacter baumannii*. β-lactamases. Resistência bacteriana.

# *Acinetobacter baumannii*: Dissemination and evolution of beta-lactamases in three hospitals of northern Parana during 20 years


## *ABSTRACT*

The antimicrobial resistance, in particular *Acinetobacter baumannii* (Ab) has become a serious problem worldwide public health and the production of β-lactamases are the principal mechanism involved. Thus the study assessed the evolution of β-lactamases and susceptibility to β-lactam antibiotics in Ab isolated over the past 20 years. Samples of Ab were selected from different patients admitted to intensive care unit of three hospitals in northern Parana in the period 1994-2014. To analyze the genotyping of samples from different periods, it used the technique of Enterobacterial Repetitive Intergenic Consensus - polymerase chain reaction (ERIC-PCR). Genes of carbapenemases (*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{OXA-143}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{NDM}}$ and *bla*$_{\text{KPC}}$) and extended-spectrum β-lactamases (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{PER}}$, *bla*$_{\text{GES}}$ *and bla*$_{\text{VEB}}$) were detected by PCR-multiplex. Susceptibility testing for different groups of antimicrobial agents was determined by agar dilution. Among the 176 samples found, 84.6% (149/176) were resistant to carbapenems. When analyzed by periods, resistance to imipenem and meropenem rate increased from 0% in the period 1994-1996 to 66.7% in 2004 and 100% in 2014. The reduction in susceptibility was accompanied with the evolution of the presence of the gene *bla*$_{\text{OXA-23}}$, which was 0% in 1994 to 98.4% in 2014, already *bla*$_{\text{OXA-51}}$ gene was identified in 100% of isolates. In an unprecedented way in Brazil, this study reported the presence of the gene *bla*$_{\text{CTX-M-15}}$ (Genbank KT945131) in four isolates Ab carrying gene *bla*$_{\text{OXA-23}}$ belonging to two clones, in which one was found in two hospitals at different times (2009 and 2014). The susceptibility to polymyxin B was 100% and 10% on average cephalosporins. The other genes searched (carbapenemase and ESBL) were not detected in any isolated. The genotyping showed the presence of 92 clusters among them, 11 were detected by more than one period in different hospitals. The results showed an increase of isolated carrying *bla*$_{\text{OXA-23}}$ in the last twenty years, consequently increasing resistance to carbapenems. These data and the detection of CTX-M-15 reinforces the need to implement surveillance measures to control the dissemination of Ab.



***Keywords:*** *Acinetobacter baumannii*. β-lactamases. Bacterial resistance.

# LISTA DE ILUSTRAÇÕES

## Capítulo 1



## Capítulo 2

### Manuscrito 1



### Manuscrito 2

Dissertação elaborada e formatada conforme as normas da publicação científica: ***Journal of Hospital Infection*** (manuscrito 1) disponível em: http://www.elsevier.com/journals/ajic-american-journal-of-infection-control/0196-6553/guide-for-authors

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# SUMÁRIO

# CAPÍTULO I

## 1. Introdução:

A descoberta da penicilina por Alexander Fleming em 1928 abriu uma nova era no tratamento de doenças infeciosas, e por mais de 60 anos, os antimicrobianos foram consideradas “drogas milagrosas” para curar as infecções [1].

Até os anos 1970, muitos novos antimicrobianos foram desenvolvidos para que os patógenos mais comuns fossem totalmente susceptíveis. Entretanto após os anos de 1990 o cenário mudou completamente, e poucos são os investimentos para novos fármacos dessa categoria. (Figura 1) [2].

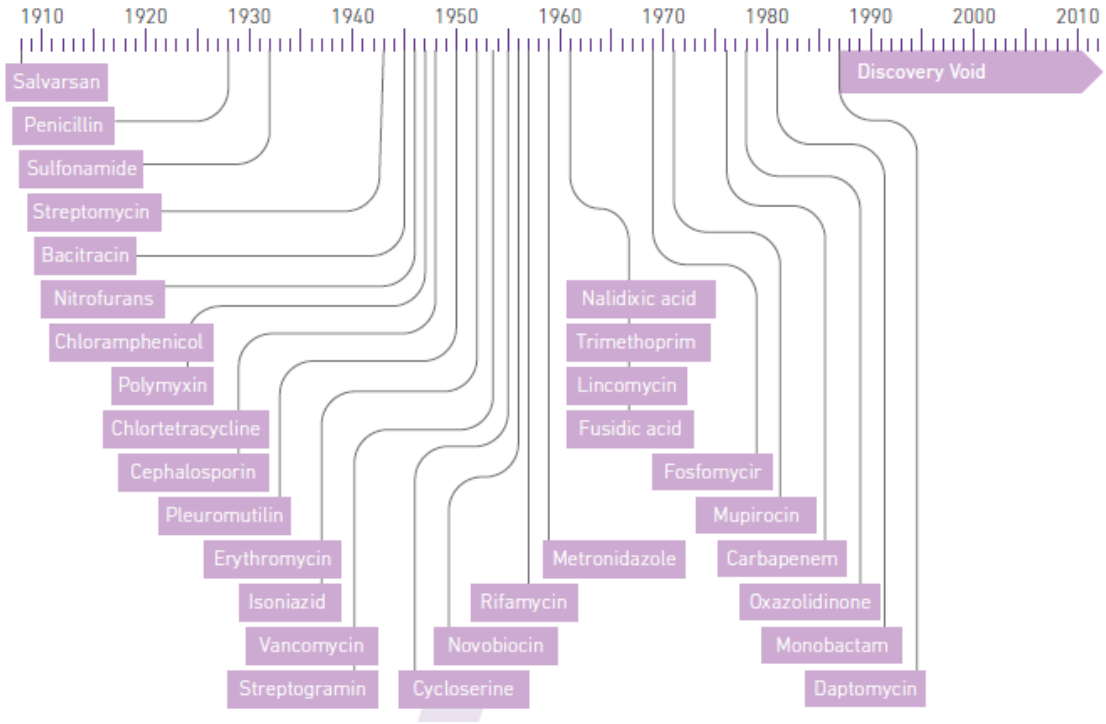


**FIGURA 1.** Períodos de descobertas de distintas classes de antimicrobianos. Fonte: Antimicrobial resistance: global report on surveillance, World health organization, 2014.

Logo após a introdução da penicilina, cepas bacterianas resistentes foram detectadas, e a rápida evolução da resistência aos antimicrobianos resultou nos últimos anos uma das mais graves ameaças globais para a saúde humana no século XXI [3].

O desenvolvimento da resistência antimicrobiana é um fenômeno natural em microrganismos, mas pode ser acelerada pela pressão seletiva exercida pelo uso e abuso de agentes antimicrobianos na medicina humana, agricultura e veterinária [4].

Um recente estudo realizado por Boeckel e colaboradores (2014), avaliou os padrões de consumo de antimicrobianos entre os anos de 2000 a 2010 para 16 grupos de medicamentos em 71 países. Para a análise foram utilizados dados de vendas de antimicrobianos para varejo e farmácias hospitalares, considerando o número de doses vendidas como um comprimido, cápsula, ou ampola registrada em um banco de dados do *Intercontinental Marketing Services of Health - IMS'Health* (Danbury, CT, EUA). Os dados demonstraram que houve um aumento de 36% do consumo, destacando os países em desenvolvimento como Brasil, Rússia, Índia, China e África responsáveis por 76% desse aumento. Diversas classes de antimicrobianos apresentaram um significativo aumento do consumo, como as cefalosporinas, monobactâmicos, glicopeptídeos, fluorquinolonas, carbapenêmicos e polimixinas [(5)].

Infecções hospitalares, especialmente causadas por bacilos Gram-negativos multirresistentes (BGN-MR), constituem uma epidemia global, principalmente entre os pacientes internados nas unidades de terapia intensivas (UTIs), incluindo predominantemente: *Acinetobacter baumannii, Pseudomonas aeruginosa* e enterobactérias [(6)].

O aumento da resistência em microrganismos, tais como os BGN-MR, é de grande preocupação na saúde pública, uma vez que existem poucas opções terapêuticas eficazes no tratamento das infecções, e um número limitado de agentes antimicrobianos estão em desenvolvimento, para substituir os que se tornam ineficazes. Desse modo traz à urgência necessidade de proteger a eficácia de drogas existentes [(7)], e o desenvolvimento de estratégias para reduzir a disseminação da resistência antimicrobiana, e controle das infecções hospitalares [(8)].

O gênero *Acinetobacter* é constituído por 43 espécies (http://www.bacterio.net/acinetobacter.html#r, último acesso em 08 de novembro de 2015). Compreende bactérias Gram-negativas cocobacilares, não fermentadoras da glicose, catalase positiva, oxidase negativa e estritamente aeróbias [(9)].

A espécie de *Acinetobacter baumannii* é considerada a de maior importância clínica. Pertence ao complexo *Acinetobacter calcoaceticus* juntamente com *A. pittii*, *A. nosocomialis e A. calcoacetius,* sendo esse complexo diferenciado apenas por técnicas moleculares, pois são indistinguíveis pelas características fenotípicas [(9,10)].

*A. baumannii* está comumente envolvido em infecções hospitalares sendo responsável por uma variedade de infecções associadas aos cuidados de saúde, tais como: bacteremia, infecção urinária, infecção cirúrgica local e pneumonia associada à ventilação mecânica [(7,11)].

Dados do SENTRY *Antimicrobial Surveillance Program* revelaram que entre os anos de 2008 - 2010 *A. baumannii* foi considerado um dos principais patógenos responsáveis em causar pneumonia e infecções da corrente sanguínea na América latina [12].

A espécie de *A. baumannii* já foi isolada do solo, água, animais e humanos, e apresentam a capacidade de sobreviver por períodos prolongados em múltiplos reservatórios (ex. pacientes colonizados, superfícies inanimadas, reservatórios úmidos ou sob dessecação), favorecendo sua persistência e disseminação no ambiente hospitalar [9,10].

A resistência intrínseca apresentada a diferentes classes de antimicrobianos, somada a sua habilidade em adquirir e acumular elementos genéticos móveis, com determinantes de resistência adicionais, também pode ser considerado uma das principais vantagens de sobrevivência de *A. baumannii* [11].

Os carbapenêmicos (imipenem e meropenem) são antimicrobianos β-lactâmicos de amplo espectro e alta potência contra bacilos Gram-negativos. Foram introduzidos na década de 80, e desde então tem sido considerados as principais opções terapêuticas para o tratamento de infecções causadas por *A. baumannii*. Entretanto nos últimos anos, relatos de resistência a esses fármacos e surtos hospitalares causados por *A. baumannii* resistente a carbapenêmicos, tem sido observados em diversas partes do mundo, inclusive no Brasil. Restringindo o tratamento das infecções causadas por esse patógeno, a novos antimicrobianos como a tigeciclina ou ao uso das polimixinas (poliximina B e colistina) [12 13,14].

Vários mecanismos de resistência têm sido identificados em *A. baumannii*, entre eles, hiperprodução de sistemas de efluxo, alteração nas proteínas ligadoras de penicilinas (*penicillin binding proteins* – PBPs) e modificações ou perdas de proteínas de membrana externa (porinas). No entanto, é a produção enzimática, o principal mecanismo de resistência desta bactéria devido sua facilidade de disseminação lateral para outros microrganismos, uma vez que os genes codificadores destas enzimas encontram-se geralmente localizados em plasmídeos ou transposons [15].

A produção de β-lactamases, em especial do tipo carbapenemase (i.e. β-lactamases com capacidade de degradação aos carbapenêmicos) são os tipos mais comum e preocupante [16].

As carbapenemases têm sido identificadas desde a introdução do imipenem na década de 1980, porém nos últimos 10 anos, houve um aumento substancial no relato dessas enzimas [17].

Em 1980, Ambler propôs a classificação das enzimas β-lactamases em quatro importantes classes moleculares, baseando-se na estrutura proteíca primária e na identidade das suas sequências de aminoácidos [18].

- Classe A: Serino β-lactamases, incluindo as β-lactamases de espectro ampliado (ESBL), penicilinases e carbenicilinases.

- Classe B: Metalo- β-lactamases

- Classe C: Cefalosporinases cromossomais

- Classe D: Oxacilinases

Bush (1989) propôs uma segunda classificação baseando no substrato preferencial e propriedades inibitórias à estrutura molecular da enzima[19,20]. Em 1995 Bush, Jacoby e Medeiros sugeriram uma atualização da classificação inicial de Bush que combinavam as caracterísitcas estruturais e funcionais das β-lactamases[21]. Em 2010, esta mesma classificação foi atualizada, de modo a incluir novas β-lactamases descritas a partir de 1995 [22].

A Tabela 1 apresenta de modo simplificado, a correlação entre as três classificações e as características funcionais das β-lactamases.

As carbapenemases descritas em *A. baumannii* incluem as OXA-carbapenemases, as metalo-β-lactamases (MβL), *Klebsiella pneumoniae* carbapenemase (KPC), e Guiana *extended spectrum* carbapenemase (GES-carbapenemase) [15, 21, 23].

### 1.1. Carbapenemases:

#### 1.1.1 Oxacilinases

A classe do tipo oxacilinases (OXA), também conhecidas como carbapenemases da classe D de Ambler (1980) (capazes de hidrolisar carpanemêmicos CHDL – c*arbapenem-hydrolyzing class D* β-lactamases), ou pertencentes aos grupos 2d, 2de, 2df segundo Bush e Jacoby (2010) é considerada a mais disseminada no Brasil e no mundo, com mais de 160 variantes descritas na literatura [12]. A denominação desta classe se deve a alta atividade hidrolítica apresentada contra oxacilina, cloxacilina e meticilina [21].

Geralmente não hidrolisam cefalosporinas de amplo espectro e aztreonam, e possuem baixa atividade hidrolítica contra o imipenem e meropenem, entretanto muitas vezes

apresentam-se associados com elementos de inserção, que consequentemente podem aumentar a expressão de carbapenemases [23].

Atualmente em *A. baumannii* as OXAs são divididas em seis principais subgrupos filogenéticos, onde cinco deles correspondem à CHDls adquiridas, geralmente identificados em plasmídeos, transposons ou associados a sequências de inserção, o que pode ajudar a explicar sua maior facilidade de disseminação [16, 24].

As CHLs adquiridas são: OXA-23-*like* (variantes OXA-23, -27, - 49, -102, -103, -105, -133, -146, -165 a -171), OXA-24/33/40-*like* (variantes OXA-24/33/40, -25, -26, -72, -139, -160, -182), OXA-58-*like* (variantes OXA-58, -96, -97, -164), OXA-143 (variantes OXA-143 e -231) e OXA-235-*like.* O Um último subgrupo, OXA-51-*like* corresponde às carbapenemases de ocorrência natural ou intrínseca em *A. baumannii,* geralmente localizados no cromossomo [16, 24, 25, 26].

Os subgrupos de OXA-23, OXA-24, OXA-51 e OXA-58-*lik*e estão disseminados em diversas regiões geográficas do mundo, enquanto a OXA-143-like ainda encontram-se restrita no Brasil, especialmente na região sudeste, e a OXA-235 nos EUA e México, pois foram recentemente descritas nesses países [27, 28, 29].

Os genes codificadores do grupo OXA-24/33/40 são assim denominados, pois essas três variantes possuem sequências aminoácidos idênticos, mas foram atribuídos nomes diferentes no GenBank® [16].

A primeira OXA foi identificada em 1985, a partir de um isolado multirresistente de *A. baumannii* na Escócia. A princípio foi designado ARI-1 (*Acinetobacter* resistente ao imipenem), posteriormente foi denominada como OXA-23 [30].

No Brasil, o primeiro relato do gene $bla_{\text{OXA-23}}$ ocorreu em um surto em 2003 na cidade de Curitiba – Paraná, após 14 anos da primeira identificação descrita na Escócia [31].

E atualmente este gene tem sido encontrado em isolados de *A. baumannii* de diversas regiões do Brasil e do mundo [17], muitas vezes associados a surtos hospitalares [32].

**Tabela 1**. Características funcionais e moleculares dos principais grupos de β-lactamases

| Classificação de Bush e Jacoby, 2010 | Classificação de Bush, Jacoby, Medeiros, 1995 | Classificação de Ambler, 1989 | Características Funcionais | Enzimas |
|---|---|---|---|---|
| 1 | 1 | C | Hidrolisa cefalosporinas e cefamicinas, geralmente com valores maiores de $k_{cat}$ quando comparadas às penicilinas.<br>Não inibida pelo ácido clavulânico e tazobactam.<br>Alta afinidade por aztreonam. | AmpC de *Pseudomonas aeruginosa* e *Escherichia coli*, CMY-2, FOX-1, MIR-1, P99 |
| 1e | NI | C | Hidrolisa penicilinas, cefamicinas, cefalosporinas de espectro ampliado e monobactâmicos.<br>Não inibida pelo ácido clavulânico e tazobactam. | GC1, CMY-37 |
| 2ª | 2ª | A | Hidrolisa eficientemente as penicilinas.<br>Inibida pelo ácido clavulânico e tazobactam. | PC1 e outras penicilinases de *Staphylococcus* spp |
| 2b | 2b | A | Hidrolisa eficientemente as penicilinas, a cefaloridina, a cefazolina e a cefalotina.<br>Inibida pelo ácido clavulânico e tazobactam. | SHV-1, TEM-1, TEM-2<br>TEM-90 |
| 2be | 2be | A | Hidrolisa penicilinas, cefalosporinas de espectro ampliado e monobactâmicos.<br>Inibida pelo ácido clavulânico e tazobactam. | ESBLs: CTX-M-15, PER-1, SFO-1, SHV-5, TEM-10, TEM-26, VEB-1 |
| 2br | 2br | A | Hidrolisa eficientemente as penicilinas, a cefaloridina, a cefazolina e a cefalotina.<br>Inibida fracamente pelo ácido clavulânico. | IRTs: TEM-30, TEM-76, TEM-103, SHV-10, SHV-26 |
| 2ber | NI | A | Hidrolisa penicilinas, cefalosporinas de espectro ampliado e monobactâmicos.<br>Menos eficientemente inibida pelo ácido clavulânico e tazobactam. | CMTs: TEM-50, TEM-68, TEM-89 |
| 2c | 2c | A | Hidrolisa eficientemente a carbenicilina.<br>Inibida pelo ácido clavulânico. | PSE-1, CARB-3 |
| 2ce | NI | D | Hidrolisa eficientemente carbenicilina, cefepima e cefpiroma.Inibida pelo ácido clavulânico e tazobactam. | RTG-4 |

**Tabela 1**. Continuação

| Classificação de Bush e Jacoby, 2010 | Classificação de Bush, Jacoby, Medeiros, 1995 | Classificação de Ambler, 1989 | Características Funcionais | Enzimas |
|---|---|---|---|---|
| 2d | 2d | D | Hidrolisa eficientemente a cloxacilina ou oxacilina. Inibição variável pelo ácido clavulânico. | OXA-1, OXA-10 |
| 2de | NI | D | Hidrolisa penicilinas e cefalosporinas de espectro ampliado. Inibição variável pelo ácido clavulânico. | ESBLs: OXA-11, OXA-15 |
| 2df | NI | D | Hidrolisa carbapenens e cloxacilina ou oxacilina. Inibição variável pelo ácido clavulânico. | OXA-23, OXA-48 |
| 2e | 2e | A | Hidrolisa eficientemente cefalosporinas. Inibida pelo ácido clavulânico e tazobactam. | CepA |
| 2f | 2f | A | Hidrolisa carbapenens, cefalosporinas, penicilinas e cefamicinas. Fracamente inibida pelo ácido clavulânico e tazobactam | IMI-1, KPC-2, KPC-3, SME-1, GES-2 |
| 3ª | 3 | B | Hidrolisa todos os antimicrobianos β-lactâmicos. Inibida pelo EDTA e quelantes de íons divalentes, não Inibida pelo ácido clavulânico e tazobactam. | IMP-1, L1, NDM-1, VIM-1 |
| 3b | 3 | B | Hidrolisa preferencialmente carbapenens. Inibida pelo EDTA e quelantes de íons divalentes, não Inibida pelo ácido clavulânico e tazobactam. | CphA, Sfh-1 |
| NI | 4 | ND | Enzimas não sequenciadas que não são agrupados em outros grupos. | |

Abreviatura: NI, grupo não incluso; ND, não determinado (Tabela retirada da tese de doutorado de Adriana Giannini Nicoletti, UNIFESP – 2014 [33] que foi adaptada do artigo de Bush & Jacoby, 2010 e Bush & Fisher, 2011).

### 1.1.2. *Klebsiella pneumoniae carbapenemase*

A *Klebsiella pneumoniae carbapenemase* (KPC), conhecida popularmente como "super bactéria", é uma carbapenemase pertencente à classe A de Ambler (1980) e ao grupo 2f de Bush, Jacoby e Medeiros (1995). O primeiro relato (*bla*$_{KPC-1}$) ocorreu em 2001 em isolados de *K. pneumoniae* resistentes aos carbapenêmicos na Carolina do Norte, Estados Unidos [34]. Logo descrições de variantes foram identificadas como *bla*$_{KPC-2}$, entretanto uma revisão na sequência da enzima KPC-1 revelou que a mesma apresentava 100% de similaridade com a KPC-2, consequentemente considerou-se inválida a designação KPC-1[35].

Atualmente existem 23 variantes de KPC descritas que se diferenciam entre si por alterações de aminoácidos (www.lahey.org/studies, último acesso em novembro de 2015). Esta carbapenemase é capaz de hidrolisar penicilinas, cefalosporinas, aztreonam e carbapenêmicos, e é fracamente inibida pelo ácido clavulânico e pelo tazobactam [34]. No Brasil, a presença da enzima KPC foi relatada pela primeira vez em 2009, em isolados de *K. pneumoniae* no estado de Recife [36]. E desde então uma grande disseminação do gene *bla*$_{KPC-2}$ vem sendo observada no país, em diversas regiões. Entretanto, a disseminação desse gene inclui predominantemente espécies da família *Enterobacteriaceae* e não fermentadores da glicose como *Pseudomonas aeruginosa* [37]. Relatos de *A. baumannii* produtores de KPC ainda são restritos a Porto Rico [38].

### 1.1.3. GES-carbapenemase

GES-1 (*Guiana extended spectrum*) foi inicialmente descrita em *K. pneumoniae* em um hospital da Guiana Francesa em 1998. Devido sua atividade hidrolítica contra penicilinas e cefalosporinas de amplo espectro, foi classificada como uma β-lactamases de espectro estendido (ESBL) [4].

Em 2000, na África dos Sul, uma nova variante (GES-2) foi descrita em uma *P. aeruginosa*, com a alteração de aminoácidos o espectro de ação passou a incluir hidrólise dos carbapenêmicos, consequentemente foram incluídas na classe de carbapenemases [39]. Em *A. baumannii* as variantes mais encontradas são GES-11, -12 e -14, entretanto ainda são poucos os relatos identificados neste microrganismo [40].

### 1.1.4. Metalo-β-lactamases

As Metalo-β-lactamases (MβL) pertencem à classe B de Ambler (1980) e ao grupo 3a e 3b segundo Bush, Jacoby e Medeiros (1995). A denominação desta classe se deve ao fato de utilizarem um ou mais íons zinco ($Zn^{+2}$) ou outros cátions divalentes no seu sítio ativo para calalisar a hidrólise do anel β-lactâmico, por sua vez são inibidas por quelantes metálicos, como o ácido etilendiaminotetraacético (EDTA) [41,42].

As MβL são capazes de hidrolisar todas as classes de β-lactâmicos, exceto os monobactâmicos, e constitui o grupo mais relevante de carbapenemases encontrado em enterobactérias, *Acinetobacter* spp. e principalmente em *P. aeruginosa* [43]. Até o momento várias famílias já foram descritas, sendo as enzimas IMP (imipenemase), VIM (Verona imipenemase), e NDM (*New Delhi* imipenemase), consideradas as principais representantes e estão amplamente disseminadas [44]. Enquanto SPM (São Paulo metallo-β-lactamase), GIM (German imipenemase), SIM (Seul imipenemase), AIM *(*Australian imipenemase), KHM (Kyorin Health Science MBL), DIM (Dutch imipenemase), TMB (Tripoli metallo-β-lactamase) estão restritas a determinadas regiões geográficas [14, 42, 45,46].

O surgimento de genes que codificam as MβL é preocupante, uma vez que eles geralmente estão inseridos em estruturas genéticas móveis com grande capacidade de disseminação. Por isso, a detecção precoce de microrganismos produtores de MβL é crucial para estabelecer terapia antimicrobiana adequada e para impedir a disseminação hospitalar [43].

Em *A. baumannii* as MβL não são comumente encontradas, e quando identificadas os principais grupos relatados são as do tipo SIM, IMP, VIM e a mais recentemente identificada NDM [15]. No Brasil a presença de MβL do tipo IMP foi descrita como principal mecanismo de resistência em isolados de Hospital São Paulo entre os anos de 1998 a 2003 [47], no entanto este mecanismo de resistência diminuiu gradualmente sendo substituído pela produção de OXA carbapenemase que hoje é o maior problema em *A. baumannii* do Brasil.

### 1.2. β-lactamases de espectro estendido

A introdução de cefalosporinas de espectro ampliado no início de 1980 na prática clínica representou um grande avanço para o tratamento de infecções causadas por espécies da família de *Enterobacteriaceae* e outros agentes patogênicos Gram-negativos. Entretanto, ao mesmo tempo, o uso extensivo de cefalosporinas gerou uma pressão seletiva que foi seguido

pelo rápido aparecimento de novas β-lactamases que conferem resistência a estes compostos, denominadas β-lactamases de espectro estendido (*Extented spectrum* β-lactamases- ESBL) [48].

As ESBL são categorizadas como classe A de Ambler por conter serina em seu sítio ativo. Estas enzimas são capazes de hidrolisar cefalosporinas de terceira (ceftazidima, cefotaxima, ceftriaxona) e quarta geração, (cefepime), monobactâmicos (aztreonam), e são inativadas por compostos como clavulanato, sulbactam e tazobactam. Até o momento uma variedade destas β-lactamases já foram caracterizadas e muitas descritas no Brasil [49].

A presença de ESBL ocorre predominantemente em espécies da família *Enterobacteriacea,* especialmente em *K. pneumoniae* e *Escherichia coli,* mas também são encontradas em não fermentadores, como *P. aeruginosa e A. baumannii* [4,50].

Os genes de ESBL geralmente são codificados por plasmídeos, o que facilita a disseminação e a transferência horizontal para outras espécies bacterianas. Frequentemente, na mesma cepa, podem existir vários plasmídeos e múltiplas ESBLs. Estes plasmídeos podem codificar também outros genes de resistência antimicrobiana. Desta forma, é comum que microrganismos expressam uma ESBL com co-resistência, por exemplo, aos aminoglicosídeos, tetraciclinas, cloranfenicol e outros antimicrobianos [51].

### 1.2.1. Família TEM, SHV e CTX-M

TEM-1 foi a primeira β-lactamase descrita em bactérias Gram-negativas, encontrada em um isolado de *E. coli* recuperada de uma amostra de uma paciente chamada Temoniera na Grécia em 1965, por isso a designação TEM. Após alguns anos do primeiro isolamento, TEM-1 já estava disseminada no mundo. Logo substituições de aminoácidos na enzima deram origem a diversas variantes da família [52].

Derivada de uma propriedade bioquímica da TEM, originou SHV-1, com variável sulfidrila (***S**ulp**h**ydryl **V**ariable)* pela substituição de um ou mais aminoácidos, sendo comumente encontrada em *K. pneumoniae,* conferindo resistência às penicilinas de amplo espectro tais como a ampicilina, tigeciclina, piperacilina, e cefalosporinas [4].

Até o final da década de 1990, as enzimas TEM e SHV eram as mais comumente encontradas em infecções causadas por bactérias Gram- negativas. Em 1989, foi relatado um

novo membro da classe das ESBL denominado CTX-M (*cefotaximases*) e partir do ano 2000, estas se tornaram as ESBL mais comumente detectadas [53]. Estas enzimas apresentaram maior atividade contra cefotaxima em relação à ceftazidima, e melhor inibição pelo tazobactam, em relação ao ácido clavulânico [54, 55].

Enzimas CTX-M apresentaram uma elevada similaridade com as sequências de genes de β-lactamase cromossômicas de espécies *Kluyvera georgiana* encontradas no solo, sugerindo que esta família de ESBL, pode representar variantes genéticas descendentes de espécies de *Kluyvera* [50, 56].

Atualmente existem 172 variantes de CTX-M (http://www.lahey.org/Studies último acesso em novembro de 2015), originadas a partir de seis principais grupos (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 e KLUC), com a maioria de variantes encontradas no grupo CTX-M-1 e 9 [48]. Dentre elas, destacam-se as ESBL dos tipos CTX-M-14 e CTX-M-15, como as mais disseminadas no mundo todo, especialmente CTX-M-15 tem sido relatada cada vez mais em ambientes comunitários e hospitalares em país europeu, africano, asiático e em países sul e norte americano [48, 56].

A presença de CTX-M encontra-se bem difundida em enterobactérias e raramente em *A. baumannii,* com relatos apenas no Japão com a presença de CTX-M-2 [57] e algumas CTX-M-15- descrita na India [58] e Haiti [59].

No Brasil, CTX-M-15 foi reportada pela primeira vez em 2006, em isolados clínicos de *E. coli* de um hospital no estado de São Paulo e mais tarde foi relatada em *K. pneumoniae* no Rio de Janeiro [60]. E estão se tornando cada vez mais prevalente em infecções associadas à comunidade, e curiosamente foi relatado que a carne de frango possui um potencial para fonte de bactérias multirresistentes que transportam inclusive ESBL. Um estudo realizado no Rio de Janeiro observou carne de frango contaminado com *E. coli* produtoras de CTX-M-15. Este fato é preocupante, pois muitos estudos apontam que estes microrganismos podem ser transmitidos para os seres humanos, e casos similares já foram observados na Europa e na Ásia [61].

### 1.2.2. Família VEB e PER

As ESBL do tipo PER (*Pseudomonas extended resistance*), compartilham cerca de 25-27% de homologia dos tipos TEM e SHV. A PER-1 foi detectado pela primeira vez em 1991

na França em um isolado de *P. aeruginosa* e mais tarde em *Salmonella enterica serovar Typhimurium*e isolados de *Acinetobacter* spp. Esta β-lactamases hidrolisa eficientemente penicilinas e cefalosporinas e é susceptível a inibição do ácido clavulânico. Na Turquia 46% dos isolados hospitalaraes de *Acinetobacter* spp e 11% de *P. aeruginosa* foram encontrados produzindo PER-1 [4, 62].

Em 1996, a VEB-1 (*Vietnamese extended spectrum* β-lactamase) foi identificada pela primeira vez em uma *E. coli* isolada no Vietnã, após a detecção disseminou por muitos países em membro da família *Enterobacteriaceae* [54, 63]. A enzima VEB-1 confere resistência de alto nível para ceftazidima, cefotaxima, e aztreonam, e pode ser inibidas pelo clavulanato [54].

Em 2003 na França, ocorreu o primeiro relato de VEB-A em *A. baumannii* [64]. Logo outros relatos ocorreram na Bélgica, Argentina, Irãn, Taiwan [15].

## 2. JUSTIFICATIVA

A capacidade de *A. baumannii* adquirir mecanismos de resistência aos antimicrobianos possibilitou a este microrganismo persistir no ambiente hospitalar, bem como facilitou o surgimento de cepas multidrogas resistentes.

Atualmente, já existem relatos sobre infecções causadas por isolados extensivamente resistentes, ou seja, apresenta resistência a todos os antimicrobianos utilizados clinicamente, o que demonstra a necessidade do desenvolvimento de estratégias para a prevenção e tratamento de infecções causadas por este organismo.

Nos últimos anos altas taxas de resistência aos carbapenêmicos foram observadas no Paraná. Estudos realizados pelo nosso grupo de pesquisa detectaram um aumento da resistência em *A. baumannnii* aos carbapenêmicos de 2% (1994-1996) para 73% (2004-2007) em isolados do Hospital Universitário de Londrina e altas taxas de resistência aos carbapenêmicos em isolados do Hospital Universitário de Maringá. A presença de oxacilinases tem sido o principal mecanismo de resistência aos carbapenêmicos disseminado pelo Brasil, e particularmente no Paraná. Entretanto informações a respeito da evolução destas β-lactamases em amostras de *A. baumanii* ao longo dos anos ainda são escassas.

Diante do que foi exposto e da recente emergência de infecções causadas por cepas de *Acinetobacter* spp. multirresistentes, é de grande importância que sejam realizados estudos para que se entenda a evolução da resistência aos β-lactâmicos deste importante patógeno com o objetivo de contribuir na prevenção da sua disseminação bem como o controle microrganismo.

# 3. OBJETIVOS

## 3.1. GERAL:

- Avaliar a evolução das β-lactamases e a susceptibilidade aos β-lactâmicos ao longo dos últimos 20 anos em amostras de *A. baumannii* isolados de três hospitais da região norte do Paraná.

## 3.2. ESPECÍFICOS:

- A partir de uma coleção de microrganismos estocados no laboratório de microbiologia, selecionar amostras de *A. baumannii* de pacientes hospitalizados em unidade terapia intensiva de diferentes centros médicos. Considerando um isolado por paciente durante o período de 1994 a 2014.

- Analisar a genotipagem entre as amostras dos diversos períodos utilizando a técnica de *Enterobacterial Repetitive Intergenic Consensus* - reação de cadeia da polimerase (ERIC-PCR).

- Confirmar a Concentração Inibitória Mínima (CIM) aos carbapenêmicos (imipenem e meropenem), cefalosporinas (ceftadizima, ceftriaxona, cefepima) e polimixina B dos isolados por métodos de ágar diluição.

- Pesquisar a presença de genes codificadores de β-lactamases das classes A, B e D pela técnica de reação em cadeia da polimerase (PCR).

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# CAPÍTULO II

**Manuscrito 1:** “First report of CTX-M-15-producing *Acinetobacter baumannii* in Brazil”

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Letter to the Editor

# First report of CTX-M-15-producing Acinetobacter baumannii in Brazil

Sir,

Extended-spectrum b-lactamases (ESBLs) are bacterial enzymes that impart resistance to advanced-generation cephalosporins. The CTX-M type, specifically CTX-M-15, is the most prevalent class of ESBL.[1] In Brazil, CTX-M-15 was first reported in 2010 in clinical isolates of Escherichia coli from a hospital in São Paulo state, and later in Klebsiella pneumoniae isolates, apparently disseminated solely among *Enterobacteriaceae*.[2]

Despite the high prevalence in *Enterobacteriaceae*, this gene is rarely described in *Acinetobacter baumannii*. To the best of our knowledge, it has only been reported in India in 2010[1] and in Haiti in 2011.[3] This letter reports the third finding of CTX-M-15 production in carbapenem-resistant *A. baumannii* in the world, and the first detection in Brazil.

The isolates were obtained from different patients in the intensive care units of two hospitals in Maringá, Paraná, Brazil. In Hospital A (N ¼ 2), one isolate was recovered in November 2009 from urine, and another isolate was recovered in December 2009 from tracheal secretions. In Hospital B (N ¼ 2), one isolate was recovered in May 2011 from a hemoculture, and another isolate was recovered in August 2014 from a rectal swab. All species were confirmed using conventional techniques and an automated system.

This study was approved by the Human Ethics Committee of the Universidade Estadual de Maringá (COPEP - 621/2011).

Genotypic comparison by enterobacterial repetitive intergenic consensus[4] showed that the isolates belonged to two clones. Isolates Sc Ac 17, Sc Ac 20 (Hospital A) and HUM KPC 665 (Hospital B) showed clonal identity (Figure 1).

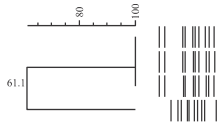
The presence of the $bla_{CTX-M}$ gene was determined by multiplex polymerase chain reaction (PCR).[5] In addition to $bla_{CTX-M}$, all of these isolates carried $bla_{OXA-51}$ and *ISAba1* upstream of the $bla_{OXA-23}$ gene. The genomic sequence of positive PCR products was determine by BigDye Terminator v3.1 (Applied Biosystems, Waltham, MA, USA). The sequence analysis and alignment results were compared with sequences available from GenBank, with 99% identity for $bla_{CTX-M-15}$ (amplicon of 593 bp).

All isolates were negative for the other carbapenemases ($bla_{OXA-24}$, $bla_{OXA-58}$, $bla_{OXA-143}$, $bla_{IMP}$, $bla_{VIM}$, $bla_{GIM}$, $bla_{SPM}$, $bla_{SIM}$, $bla_{NDM}$ and $bla_{KPC}$)[6,7] and ESBLs ($bla_{SHV}$, $bla_{PER}$, $bla_{VEB}$ and $bla_{GES}$).[5,8]

Susceptibility testing was performed by the agar dilution method, and the results were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute M100-S25.[9] The *A. baumannii* isolates were resistant to the carbapenems imipenem and meropenem, and to the cephalosporins cefepime, ceftazidime and ceftriaxone. All isolates showed susceptibility to polymyxin B.

This letter reports the first detection of $bla_{CTX-M-15}$ in multi-resistant *A. baumannii* carrying the $bla_{OXA-23}$ gene among isolates taken in 2009, 2011 and 2014 from two hospitals in Brazil.

The emergence and spread of ESBL-producing *A. baumannii* strains are worrisome as this micro-organism is considered to be a potent disseminator of the resistance gene.




| Isolates | Date | Origin | Site | IPM | MEM | FEP | CAZ | CRO | PMB | *bla*CTX-M-15 | ISAba1/ $bla_{OXA-23}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HUM KPC 665 | Aug/14 | B | Rectal swab | 32 (R) | 32 (R) | 128 (R) | >256 (R) | >512 (R) | 0.25 (S) | + | + |
| Sc Ac 17 * | May/09 | A | Urine | 8 (R) | 16 (R) | 256 (R) | >256 (R) | >512 (R) | 1 (S) | + | + |
| Sc Ac 20 | Nov/09 | A | Tracheal secretion | >64 (R) | 32 (R) | 128 (R) | >256 (R) | >512 (R) | 0.25 (S) | + | + |
| HUM Ac17 | May/11 | B | Hemoculture | 32 (R) | 32 (R) | 128 (R) | >256 (R) | >512 (R) | 2 (S) | + | + |

Figure 1. Characteristics of four CTX-M-15-producing *Acinetobacter baumannii* isolates obtained in Hospitals A and B in Maringá, Paraná, Brazil. The minimum inhibitory concentrations (MICs, mg/L) of imipenem (IPM), meropenem (MEM), cefepime (FEP), ceftazidime (CAZ), ceftriaxone (CRO) and polymyxin B (PMB) were determined by the agar dilution method. The MICs (performed by an automated system) of isolates HUM KPC 665, Sc Ac 17, Sc Ac 20 and HUM Ac 17 were above the Clinical and Laboratory Standards Institute breakpoint of resistance to aminoglycosides (amikacin, gentamicin and tobramycin), fluoroquinolones (ciprofloxacin and levofloxacin), ampicillin-sulbactam and piperacillin-tazobactam. Isolates Sc Ac 17 and HUM KPC 665 were susceptible to tetracycline and trimethoprim-sulfamethoxazole, respectively. *ISAba1 gene upstream and downstream of gene $bla_{oxa-23}$.

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## Nucleotide sequence accession number

The nucleotide sequence data reported here have been deposited in the GenBank nucleotide database under accession number KT945131.

Conflict of interest statement
None declared.

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# CAPÍTULO II

**Manuscrito 2:** *Acinetobacter baumannii*: disseminação e evolução das beta-lactamases em três hospitais do norte do Paraná durante 20 vinte anos

*Título.* *Acinetobacter baumannii*: disseminação e evolução das beta-lactamases em três hospitais do norte do Paraná durante 20 vinte anos.

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Título. *Acinetobacter baumannii*: disseminação e evolução das beta-lactamases em três hospitais do norte do Paraná durante 20 vinte anos.

## Resumo

Com o aumento da resistência relatada em *A. baumannii* (Ab) o estudo avaliou a evolução das β-lactamases em amostras de Ab e a susceptibilidade aos β-lactâmicos ao longo dos últimos 20 anos. Isolados de Ab (1994 a 2014) foram recuperados de distintos pacientes de unidades terapia intensivas de três hospitais da região norte do Paraná. Para avaliar a similaridade entre as amostras foi utilizado técnica de *Enterobacterial Repetitive Intergenic Consensus* - ERIC-PCR. Genes de carbapenemases (*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-58}}$, *bla*$_{\text{OXA-143}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{NDM}}$ e *bla*$_{\text{KPC}}$) e β-lactamases de espectro estendido-ESBL (*bla*$_{\text{CTX}}$, *bla*$_{\text{SHV}}$, *bla*$_{\text{PER}}$, *bla*$_{\text{GES}}$ e *bla*$_{\text{VEB}}$) foram detectados por PCR-multiplex. Testes de susceptibilidade a diferentes grupos de agentes antimicrobianos foram determinadas por ágar diluição. O estudo demonstrou um rápido aumento e progressivo da presença do gene *bla*$_{\text{OXA-23}}$ (0% em 1994 a 98,4 em 2014) associada ao aumento da resistência aos carbapenêmicos (evoluindo de 0% em 1994 a 100% em 2014). E de maneira inédita no Brasil, reportamos a presença do gene *bla*$_{\text{CTX-M-15}}$ (Genbank KT945131), em quatro isolados de Ab carreando gene *bla*$_{\text{OXA-23,}}$ pertencentes a dois clones, nos quais um deles foi encontrado em dois hospitais em épocas diferentes (2009 e 2014). A sensibilidade a polimixina B foi de 100% e a cefalosporinas em media 10%. Genes que codificam SHV, VEB, PER, GES, KPC e MβL não foram detectados. Dentre as oxacilinases, o gene *bla*$_{\text{OXA-51}}$ foi identificado em todas as amostras e o gene *bla*$_{\text{OXA-23}}$ foi detectado em 79,5% (140/176). A genotipagem demonstrou a presença de 92 *clusters* dentre eles, 11 foram detectados por mais de um período em diferentes hospitais. Os dados demonstram que ao longo dos vinte anos analisados não houve variabilidade de β-lactamases nos isolados de Ab, entretanto, a presença do gene *bla*$_{\text{OXA-23}}$ foi frequente com grande aumento a partir de 2004 e se tornou endêmico em diferentes *clusters* resistentes aos carbapenêmicos. O achado do primeiro Ab carreando o gene *bla*$_{\text{CTX-M-15}}$ no Brasil, demonstra a necessidade urgente de medidas para o controle deste microrganismo.

**Palavras - chave:** *Acinetobacter baumannii*. β-lactamases. Resistência bacteriana.

## INTRODUÇÃO

No início da década de setenta, infecções causadas por *Acinetobacter baumannii* eram tratadas com sucesso com antimicrobianos de espectro limitado, no entanto, a habilidade em adquirir e desenvolver mecanismos de resistência tem dificultado o tratamento de infecções por este microrganismo, causando sérias complicações clínicas e altas taxas de mortalidade [1,2].

Os carbapenêmicos (imipenem e meropenem) são antimicrobianos β-lactâmicos de amplo espectro e alta potência contra bacilos Gram-negativos, desde a introdução na prática clínica foram considerados os agentes mais importantes no tratamento das infecções causadas por *A. baumannii* [3].

Entretanto a rápida emergência global de cepas de *A. baumannii* resistentes a todos os β-lactâmicos, incluindo os carbapenêmicos tem sido relatada, chegando a taxas superiores a 70% em hospitais brasileiros [4].

Vários mecanismos de resistência têm sido identificados em *A. baumannii*, como bombas de efluxo, perda de porinas e alteração do sítio de ação dos antimicrobianos, no entanto, a produção enzimática de β-lactamases é considerada o principal mecanismo envolvido, devido à facilidade de disseminação lateral [5,6].

As carbapenemases são as mais comuns, responsável pela resistência aos carbapenêmicos [3]. Desde 1991, com a primeira descrição no Japão de metalo β-lactamases (MβL) do tipo imipenemase (IMP-1) uma série de outras enzimas deste grupo tem sido descritas no mundo todo, entretanto, em *A. baumannii* ainda tem sido pouco identificada. No Brasil o primeiro relato ocorreu em 2003 sendo restrita a família IMP [7] e mais recentemente, em 2014 foi descrita o tipo NDM em *A. baumannii* [8].

Em relação às carbapenemases, do tipo oxacilinases (OXA), ao contrário do que ocorreu com as MβL em *A. baumannii*, a partir do primeiro relato em 2003, no Paraná, tem sido a carbapenemase mais disseminada no Brasil e no mundo. Estas enzimas possuem mais de 160 variantes e podem ser intrínsecas, adquiridas, cromossomicamente ou através de plasmídeos [1,9].

Em *A. baumannii*, cinco subgrupos foram relatados: OXA-51, OXA-23, OXA-24, OXA-58 e OXA-143 sendo o grupo da OXA-23 o mais importante [6].

O subgrupo OXA-51 difere dos demais, pois apresentam enzimas de ocorrência natural (intrínseca) em *A. baumannii*, ocorrendo na maioria ou em todas as cepas [3].

A KPC é outra representante das carbapenemases, com ampla disseminação em enterobactérias [10], mas em *A. baumannii* ainda encontra-se restrita a Porto Rico, onde foi

identificada pela primeira vez em 2010 [11]. As β-lactamases de espectro estendido do inglês *extended spectrum β−lactamese* (ESBL) mediadas por plasmídeos tem grande força de disseminação e tem sido encontrada predominantemente em enterobactérias [1,5]. Entretanto, apesar de poucos relatos, algumas dessas enzimas já foram identificadas em *A. baumannii* [5].

Embora existam relatos da detecção e disseminação de diferentes β-lactamases em *A. baumannii*, não é do nosso conhecimento a existência de estudo que avaliem a evolução destas enzimas ao longo do tempo, assim o objetivo deste estudo foi avaliar a evolução das β-lactamases e a susceptibilidade aos β-lactâmicos ao longo dos últimos 20 anos em amostras de *A. baumannii* isolados de três distintos hospitais da região norte do Paraná.

## MATERIAL E MÉTODOS

### 2.1. Isolados Clínicos

O presente estudo foi aprovado pelo parecer n° 621/11 COPEP- CAAE 318.0.093.000-11/ do Comitê de Ética em Pesquisa da Universidade Estadual de Maringá (UEM) - Paraná.

Foram selecionadas amostras de *A. baumannii* isoladas nos últimos 20 anos de distintos pacientes hospitalizados em unidade terapia intensivas (UTI), de três hospitais da região norte do Paraná. No período de 1994-1996, 2004 e 2007, a avaliação foi realizada apenas para isolados do hospital A (Hospital Universitário de Londrina – HU UEL), de 2009 a 2010 isolados do hospital B (Santa Casa de Misericórdia de Maringá), de 2011 a 2014 a análise foi realizada para isolados do hospital C (Hospital Universitário de Maringá – HUM). Para o ano de 2011 e 2014, dois hospitais participaram na avaliação, (A e C) e (B e C), respectivamente.

### 2.2 Identificação e testes de sensibilidade aos antimicrobianos

As amostras bacterianas foram identificadas por métodos bioquímicos convencionais e métodos automatizados: Phoenix$^{TM}$ BD (Becton and Dickinson, Sparks, USA) e Microscan® (Dade Behring, USA).

### 2.3 Tipagem Molecular

Para realização dos testes genotípicos o DNA bacteriano dos isolados foi extraído pelo método de fervura por 15 minutos seguido da centrifugação, onde 2µL do sobrenadante foram utilizados para as reações da reação em cadeia da polimerase (*polymerase chain reaction* - PCR).

A genotipagem foi realizada pela técnica de *Enterobacterial Repetitive Intergenic Consensus* - reação de cadeia da polimerase (ERIC-PCR), e a interpretação dos padrões de banda foram realizadas com auxílio do software Bionumerics® v.6,5 (Applied Maths, Sint-Martens-Latem, Belgium). Os isolados foram considerados pertencentes ao mesmo *cluster* quando o coeficiente de similaridade - Dice fosse maior ou igual a 0,93 [12].

### 2.4 Determinação da Concentração Inibitória Mínima

A técnica de ágar diluição foi realizada para confirmação da concentração inibitória mínima (CIM) dos antimicrobianos: imipenem (Merck Sharp & Dohme, Darmstadt, Germany), meropenem (AstraZeneca, London, United Kingdom ), cefepima (Brystol Myers Squibb Guayaquil, Equador), ceftazidima (Glaxo Smith Kline, London, United Kingdom), ceftriaxona (BioChimico, Instituto BioChimico Ltda, Rio de Janeiro, Brasil) e polimixina B (Sigma Aldrich, Steinheim, Germany), conforme preconizado pelo *Clinical Laboratory Institute Standard* (CLSI) M7-A10-2015 [13].

### 2.5 Detecção molecular de beta-lactamases

Técnicas de Multiplex PCR foram utilizadas para a detecção dos genes de OXAs, utilizando-se os primers de $bla_{\text{OXA-23}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-51}}$, $bla_{\text{OXA-58}}$ e $bla_{\text{OXA-143}}$ de acordo com Woodford (2006) [14], e para detecção de MβL e KPC utilizando-se os primers de $bla_{\text{IMP}}$, $bla_{\text{VIM}}$, $bla_{\text{GIM}}$, $bla_{\text{SPM}}$, $bla_{\text{SIM}}$, $bla_{\text{NDM}}$ e $bla_{\text{KPC,}}$ conforme metodologia descrita por Poirel (2011) [15].

Para pesquisa de genes que codificam ESBL, primers de $bla_{\text{CTX}}$ $bla_{\text{SHV}}$, $bla_{\text{PER}}$, $bla_{\text{GES}}$ e $bla_{\text{VEB}}$ foram utilizados conforme metodologia descrita por Shahcheraghi (2009) e Monstein (2007) [16,17].

Sequenciamento automatizado foi realizado no Centro de Pesquisa sobre o Genoma Humano e Células-Tronco da Universidade de São Paulo – USP. O resultado obtido foi comparado com as sequências de nucleotídeos depositadas no programa BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

## 3. RESULTADOS

No total de 176 amostras de *A. baumannii* (um isolado/ paciente de UTI), os sítios de isolamento mais importantes foram 33% (58/176) secreção traqueal, 21,6% (38/176) amostras

de vigilância de swab retal, 9,7% (17/176) swab oral/ nasal, 8% (14/176) hemocultura, 6,2% (11/176) ponta de cateter e 5,7% (10/176) urina.

No geral a taxa de resistência aos carbapenêmicos entre as amostras foi de 84,6% (149/176). Quando analisado por períodos, nos anos de 1994 a 1996 a taxa era de 0%; já em 2004, foi observado que 66,7% das amostras apresentavam resistência a imipenem e meropenem. O percentual encontrado em 2007, 2009, 2010, 2011, 2012, 2013 e 2014, foram respectivamente 100%, 61,9%, 73,7%, 85%, 88%, 86,8% e 100% das amostras (Figura 1).

A CIMs para as cefalosporinas mantiveram-se alta durante todo o período avaliado. Enquanto a polimixina B, todas as amostras foram susceptíveis (100%) ao longo dos anos.

Em relação à presença de carbapenemases do tipo OXA, entre as 176 amostras, 140 apresentaram o gene *bla*$_{\text{OXA-23}}$ (79,5%) e 100% *bla*$_{\text{OXA-51.}}$

Para isolados do hospital A (1994-2007) verificou-se que a frequência do gene *bla*$_{\text{OXA-23}}$ passou de 0% para 100% dos isolados. Para o hospital B (2009-2010 e 2014) as taxas verificadas foram de 81%, 42,1% e 100%, e para o hospital C (2011 a 2014) foram de 64,7%, 76% e 84,2%.

No período de 2010 a 2014, independente do hospital analisado, foi verificada uma evolução da frequência de OXA (42,1, 70%, 76%, 84,2% e 98,4%), acompanhada da percentagem de resistência aos carbapenêmicos (Figura 1).

Quando avaliado as ESBL um resultado inesperado foi observado, com a presença da enzima CTX-M (amplicon de 593bp) em quatro isolados. Sendo duas amostras de 2009 pertencentes ao hospital B, recuperada de secreção traqueal e urina, e outras duas amostras do hospital C, sendo uma isolada no ano de 2011 a partir de hemocultura, e outra amostra de vigilância coletada de swab retal em 2014.

A genotipagem demonstrou que os isolados do hospital B que carreavam o gene *bla*$_{\text{CTX-M}}$ foram considerados o mesmo *cluster* que os isolados do hospital C encontrado em 2014, que também carreavam o gene *bla*$_{\text{CTX-M.}}$ O sequenciamento automatizado dos amplicons obtidos na PCR do gene *bla*$_{\text{CTX-M}}$ foram comparados com as sequências disponíveis no banco de dados GenBank® ao qual apresentaram 99% de identidade com a CTX-M-15. A sequência de nucleotídeos obtida foi depositada no banco de dados com o número de acesso KT945131.

Todos os isolados apresentaram resultados negativos para os genes que codificam as MβL (*bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{SPM}}$, *bla*$_{\text{SIM}}$, *bla*$_{\text{NDM}}$ ), KPC, OXAs do tipo *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-58}}$ e *bla*$_{\text{OXA-143}}$, e ESBL (SHV, PER, GES e VEB).

Entre as 176 amostras, foram detectadas 92 *clusters* distintos. Dentre eles 11 (12, **15**, 33, 37, 39, 45, 47, 59, 66, 77, 82) estavam presentes em mais de um hospital em períodos

diferentes (Figura 2 e 3). Particular destaque para o *cluster* **15**, *bla*$_{\text{CTX-M-15}}$ positivo encontrado no ano de 2009 e novamente em 2014.

A análise da resistência e da evolução temporal das β-lactamases mostraram resultados similares tanto na análise considerando uma amostra por paciente (n=176) como na análise considerando um representante por *cluster* (n=92).

## 4. DISCUSSÃO

No presente estudo, demonstramos um aumento rápido e progressivo da presença do gene *bla*$_{\text{OXA-23}}$ associada ao aumento da resistência aos carbapenêmicos, e de maneira inédita no Brasil, reportamos a presença de quatro isolados carreando além de *bla*$_{\text{OXA-23}}$ o gene *bla*$_{\text{CTX-M-15}}$ em *A. baumannii*, até então descritos apenas na Índia e Haiti.

A resistência aos carbapenêmicos analisadas ao longo dos últimos 20 anos em três centros médicos do sul do Brasil, de modo geral evoluiu de 0% em 1994 a 100% em 2014. Um aumento significativo também foi observado em outros estudos com isolados de *A. baumannii* envolvendo nove hospitais de cinco estados brasileiros, onde as taxas de resistência ao imipenem foram de 12,6% no período de 1997-1998 para 71,4% em 2008-2010 [2]. Hoje muitos estudos indicam que o percentual de resistência aos carbapenêmicos aumentou gradualmente nos últimos anos na Europa, América do Norte e América Latina [10]. Este horizonte torna o tratamento de infeções por *A. baumannii* praticamente impossível, sendo necessária a utilização de drogas tóxicas como polimixinas ou ainda a associação de antimicrobianos buscando o sinergismo entre os fármacos.

Neste estudo, a redução da susceptibilidade aos carbapenêmicos foi observada com o aumento da frequência do gene *bla*$_{\text{OXA-23}}$, evoluindo de 0% (1994) a 98,4% (2014) entre as amostras. No Brasil, a enzima OXA-23 é considerada a mais disseminada em *A. baumannii*, e muitos estudos tem verificado que o aumento da resistência está relacionado com a alta disseminação de cepas policlonais de *A. baumannii* produtoras de OXA-23, geralmente associado com sequências de inserção (*ISAba1*), que promovem a expressão gênica de resistência, inclusive quando associado *upstream* ao gene cromossômico *bla*$_{\text{OXA-51}}$ [18]. A presença desses elementos móveis facilita a rápida transposição de genes de resistência antimicrobiana para outros microrganismos, dificultando a eficácia do tratamento [ 3].

Geralmente as oxacilinases diferem de outras carbapanemases, como MβL do tipo SPM, onde a disseminação ocorre com a presença de um único clone epidêmico de *Pseudomonas aeruginosa*, que tem sido detectado em diferentes regiões do Brasil contribuindo para as altas taxas de resistência aos carbapenêmicos nestes isolados. [19].

Quando avaliadas as demais β-lactamases, como as ESBL, de maneira inédita no Brasil nosso grupo de pesquisa identificou e relatou o primeiro caso de CTX-M-15 em *A. baumannii (accession number* KT945131). Os quatro isolados identificados com a presença do gene $bla_{\text{CTX-M-15}}$ também carreavam o gene $bla_{\text{OXA-23}}$.

A genotipagem demonstrou que a disseminação do gene entre os anos de 2009 e 2014 de instituições diferentes (hospital B e C) ocorreu pela presença de um único *cluster* que após cinco anos foi detectado novamente no ambiente hospitalar carreando o gene $bla_{\text{CTX-M-15.}}$

Enzimas CTX-M têm sido envolvidas em várias situações epidemiológicas, disseminadas por todos os continentes, com cepas ou plasmídeos epidêmicos, isolados de enterobactérias, particularmente *Klebsiella pneumoniae* envolvidas em infecções hospitalares e até mesmo em *Escherichia coli* de pacientes da comunidade [5,21].

Apesar da alta prevalência em enterobactérias, essa ESBL ainda é raramente identificada em *A. baumannii*, com o primeiro relato descrito em 2010 na India [20], em duas de 920 amostragem de urinas analisadas, entretanto neste estudo a relação de clonalidade entre os isolados não foi verificada. Um segundo relato foi reportado em 2011 no Haiti [21], com três isolados de *A. baumannii* positivos para CTX-M-15, obtidos a partir de duas amostras de feridas e de uma proveniente de swab retal de três vítimas do terrremoto ocorrido no país em 2010. Similar ao nosso estudo, no Haiti houve a presença de dois *clusters*, com a ocorrência do mesmo clone em dois pacientes diferentes do mesmo hospital. Estes achados, assim como os descritos no nosso estudo, demonstram uma disseminação intra e inter institucional.

Ao longo destes vinte anos analisados nenhuma amostra de nossa região apresentou genes de MβL, KPC e ESBL (PER, VEB, GES e SHV). Diferentemente de um estudo realizado em São Paulo (2006), onde a produção de IMP-1 foi verificada em isolados de *Acinetobacter* spp. e aumentou de 0% no período de 1993-1997 para 29% em 1998 e de 100% no período de 1999-2001 [7].

O resultado da tipagem molecular demonstrou uma alta variabilidade genética entre os isolados de *A. baumannii* em UTI, sendo detectados 92 clusters entre 176 isolados. Onze *clusters* foram encontrados em mais de um período em instituições diferentes (Figura 2), destacando *cluster* 15, responsável pela disseminação de CTX-M-15.

Estes resultados reforçam a necessidade de gerenciamento do uso de antimicrobianos, especialmente os carbapenêmicos, além de estabelecer medidas para controlar a expansão clonal de *A. baumannii.* Neste estudo não foi possível obter o vínculo epidemiológico das

amostras, entretanto sabemos que muitas vezes a transferência inter-hospitalar de pacientes colonizados também pode ter contribuído para a ampla disseminação [2,3].

As CIMs de cefalosporinas foram elevadas durante todos os anos analisados. No entanto uma limitação do nosso estudo é que os genes que codificam β-lactamases cromossômica do tipo AmpC ($bla_{ADC}$) não foram pesquisados, e estudos apontam que a resistência a cefalosporinas está relacionada com a presença destas enzimas, como no estudo de Mohmamed (2014) que identificou a prevalência de 100% de $bla_{ADC}$ entre as amostras de *A. baumannii* resistentes a cefepima, ceftazidima e cefotaxima [22].

Em relação ao tratamento de amostras resistentes aos carbapenêmicos, geralmente uma opção terapêutica é a polimixina B ou polimicina E (colistina). Neste estudo, como também ja verificado em outros, a sensibilidade a esta classe de antimicrobinos foi de 100%. Entretanto amostras com baixa susceptibilidade a polimixina já foram detectadas na Argentina, Brasil e no México [6,10]. Inclusive, recentemente um estudo revelou resistência a polimixina mediada por plasmídeo, MCR-1, em *Enterobacteriaceae*, embora atualmente confinado a China, MCR-1 está susceptível a disseminação global [23]. Até o momento não existem relatos deste tipo de resistência em *A. baumannii*, porém, essas descobertas enfatizam a necessidade urgente de uma ação global na luta contra as bactérias Gram-negativas-pan-resistentes, considerando que as opções terapêuticas estão cada dia mais restritas nessa batalha [23].

Concluimos que o aumento da resistência aos carbapenêmicos em *A. baumannii* em nossa região nos ultimos 20 anos foi alarmante e relacionado diretamente com a presença do gene $bla_{OXA-23}$. O relato inédito da presença de CTX-M-15 em quatro isolados, associados ao aumento da diversidade clonal verificado nos isolados de *A. baumannii*, reforçam a necessidade urgente da implementação de medidas de vigilância, principalmente quanto ao uso racional dos antimicrobianos no sentido de se evitar a disseminação destas cepas multirresistentes.

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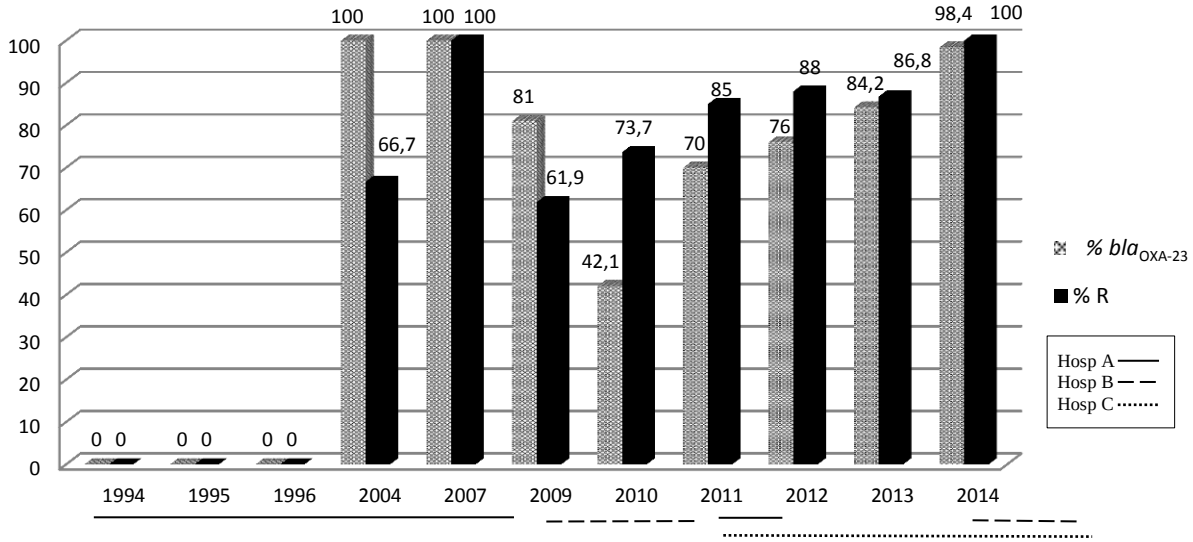
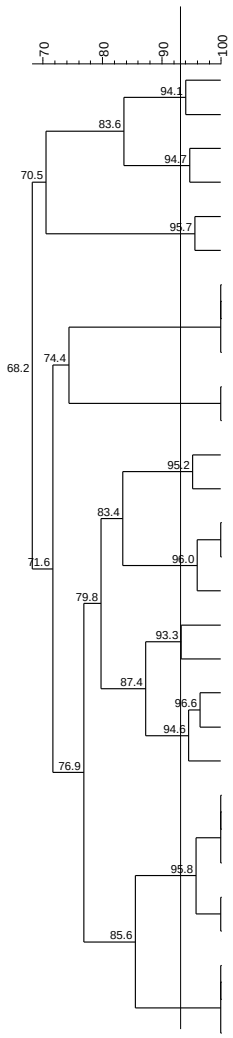



Figura 1. Evolução da resistência aos carbapenêmicos e da presença do gene $bla_{\text{OXA-23}}$ em isolados de *A. baumannii* no período de 1994 a 2014.
R= resistência; Hosp= hospital.

| Ano / *cluster* | 15* | 33 | 47 | 66 | 82 | 45 | 12 | 39 | 59 | 77 | 37 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2009 H - B | ■ | ■ | ■ | □ | □ | □ | □ | □ | □ | □ | □ |
| 2010 | □ | □ | □ | ■ | ■ | ■ | □ | □ | □ | □ | □ |
| H - B | □ | □ | □ | ■ | ■ | □ | □ | □ | □ | □ | □ |
| H - C | □ | □ | □ | □ | □ | ■ | □ | □ | □ | □ | □ |
| 2011 H - C | □ | □ | □ | □ | □ | □ | ■ | ■ | ■ | □ | □ |
| 2012 H - C | □ | ■ | □ | □ | □ | □ | ■ | ■ | ■ | ■ | ■ |
| 2013 H - C | □ | □ | □ | □ | ■ | □ | □ | □ | □ | ■ | □ |
| 2014 | ■ | □ | ■ | ■ | □ | ■ | □ | □ | □ | □ | ■ |
| H - B | □ | □ | □ | ■ | □ | □ | □ | □ | □ | □ | ■ |
| H - C | ■ | □ | ■ | □ | □ | ■ | □ | □ | □ | □ | □ |

Figura 2. Ilustração dos diferentes períodos em que os 11 *clusters* (**15**, 33, 47, 66, 82, 45, 12, 39, 59, 77 e 37) foram detectados no Hospital B e C. ******cluster* **15** responsável pela disseminação inter-hospitalar de CTX-M-15 no ano de 2009 e 2014. H = hospital.

■ Presente □ Ausente




| Isolates | Date | Initial | | Origin/ Site | MIC | | | | | | OXA | ESBL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | IPM | MEM | FEP | CAZ | CRO | PMB | | |
| HUM KPC 314 | 07-09-2013 | M.A.S | C | Rectal swab | 16 | 16 | >256 | >256 | >512 | 1 | 51,23 | |
| Sc Ac 26 | 01-22-2010 | G.N.F | B | Oral swab | 1 | 0.5 | >256 | >256 | >512 | 0.5 | 51 | |
| HUM Ac 100 | 06-20-2013 | R.G.R | C | Rectal swab | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 | |
| HUM KPC 341 | 08-28-2012 | N.M.C.S | C | Rectal swab | 32 | 32 | 256 | >256 | >512 | 2 | 51 | |
| HUM Ac43 | 11-13-2011 | R.A.S.B | C | Hemoculture | 32 | 16 | 128 | >256 | >512 | 0.25 | 51,23 | |
| HUM Ac53 | 01-23-2012 | J.R.C.S | C | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 | |
| HUM KPC 665 | 08-01-2014 | MRGS | C | Rectal swab | 32 | 32 | 128 | >256 | >512 | 0.25 | 51,23 | CTX-M-15 |
| Sc Ac 17 | 11-05-2009 | L.N.P | B | Urine | 8 | 16 | 256 | >256 | >512 | 1 | 51,23 | CTX-M-15 |
| Sc Ac 20 | 11-02-2009 | M.R.S | B | Tracheal secretion | >64 | 32 | 128 | >256 | >512 | 0.25 | 51,23 | CTX-M-15 |
| HUM Ac47 | 01-16-2012 | C.P. | C | Tracheal secretion | 32 | 32 | 64 | >256 | >512 | 2 | 51,23 | |
| HUM KPC 35 | 24-11-2011 | JCS | C | Swab retal | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 | |
| Sc Ac 64 | 05-21-2010 | R.B.S.B | B | Ora/ Nasal swabs | 2 | 2 | >256 | >256 | >512 | 0.25 | 51 | |
| Sc4 Ac94 | 09-11-2014 | J.S.M | B | Tracheal secretion | 32 | 32 | 128 | 256 | >512 | 1 | 51,23 | |
| HUM Ac75 | 08-27-2012 | J.C.L | C | Líquid abdominal cavity | 32 | 32 | >256 | >256 | >512 | 1 | 51,23 | |
| HUM Ac80 | 11-22-2012 | B.G | C | Hemoculture | 4 | 4 | >256 | >256 | >512 | 0.5 | 51 | |
| Sc Ac 6 | 08-09-2009 | S.F | B | Nasal swab | 8 | 1 | >256 | >256 | >512 | 1 | 51,23 | |
| HUM Ac27 | 07-21-2011 | M.A.G | C | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 | |
| HUM KPC 63 | 04-02-2012 | M.J.S | C | Rectal swab | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 | |
| HUM Ac72 | 07-22-2012 | L.L.A | C | Tracheal secretion | 16 | 16 | 32 | >256 | >512 | 2 | 51 | |
| Sc4 Ac9 | 04-08-2014 | H.A.A | B | Anal swab | 32 | 32 | >256 | >256 | >512 | 1 | 51,23 | |
| Sc4 Ac54 | 06-16-2014 | R.A.B | B | Anal swab | 32 | 32 | >256 | >256 | >512 | 1 | 51,23 | |
| HUM KPC 458 | 02-10-2014 | M.J | C | Rectal swab | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 | |
| HUM KPC492 | 03-10-2014 | C.G | C | Rectal swab | 32 | 32 | 128 | 256 | 256 | 1 | 51,23 | |
| HUM KPC482 | 03-10-2014 | M.F.S.L | C | Rectal swab | 32 | 32 | 128 | 256 | 256 | 1 | 51,23 | |
| HUM Ac124 | 04-07-2014 | I.A.M | C | Tracheal secretion | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 | |
| HUM KPC445 | 01-27-2014 | A.O.B | C | Rectal swab | 32 | 64 | 64 | >256 | >512 | 0.25 | 51,23 | |
| HUM KPC 661 | 07-23-2014 | S.N.A | C | Rectal swab | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 | |
| Sc Ab 29 | 06-04-2009 | P.F | B | Tracheal secretion | 8 | 8 | 256 | >256 | >512 | 1 | 51,23 | |
| Sc Ab 30 | 07-08-2009 | M.A.B | B | Tracheal secretion | 8 | 8 | 256 | >256 | >512 | 0.5 | 51,23 | |

Figura 3. Dendograma obtido pelo "*Enterobacterial repetitive intergenic consensus" – Polymerase Chain Reaction* (ERIC-PCR) dos 11 *clusters* e seus constituintes detectados em instituições e períodos diferentes. Escala representando percentual de similaridade. A linha no dendrograma denota o limiar de 93% de homologia para a definição de grupos de similaridade genética. Concentração inibitória mínima – CIM (miligramas/ litro): IPM imipenem, MEM meropenem, FEP cefepima, CAZ ceftazidima, CRO cefriaxona, PMB polimixina B; OXA oxacilinase.

# CAPÍTULO III

## CONCLUSÕES

- Um aumento rápido e progressivo da presença do gene *bla*$_{\text{OXA-23}}$ associada ao aumento da resistência aos carbapenêmicos foi verificada no últimos 20 anos em amostras de *A. baumannii*. Com a frequência do gene *bla*$_{\text{OXA-23}}$ evoluindo de 0% em 1994 a 98,4% em 2014, e a resistência de 0% em 1994 atingindo 100% em 2014.

- De maneira inédita no Brasil, reportamos a presença de quatro isolados carreando o gene *bla*$_{\text{CTX-M-15}}$ em *A. baumanii*, (*Genbank accession number* KT945131) até então descritos apenas na Índia e Haiti (**Manuscrito publicado no periódico *Journal of Hospital Infection* em dezembro de 2015).**

- Os isolados que apresentaram gene *bla*$_{\text{CTX-M-15}}$ também carreavam o gene *bla*$_{\text{OXA-23}}$ e foram detectados em duas instituições em épocas diferentes (2009 e 2014) possuindo as mesmas características genéticas. Entretanto o vinculo epidemiológico entre os mesmos não foram verificados.

- Os resultados de tipagem molecular revelaram a presença de uma grande variedade genômica (92 *clusters*) entre as amostras de *A. baumannii* (n=176). Com a presença de 11 *clusters* em mais de um período encontrado na mesma instituição de isolamento, até mesmo em outro centro médico.

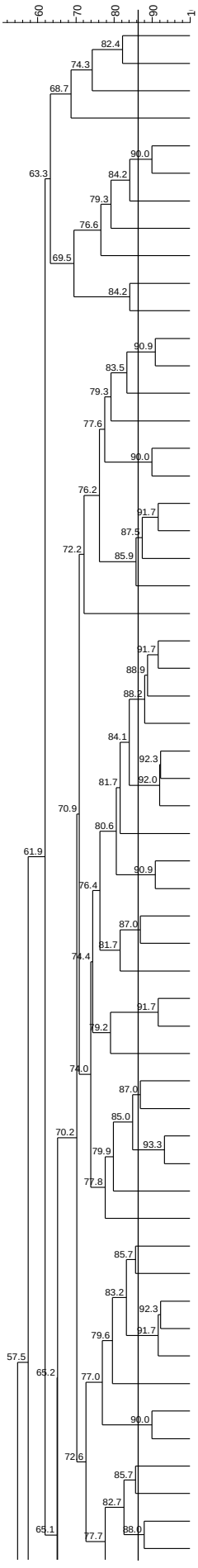
## PERSPECTIVAS FUTURAS

- Estudar mais detalhadamente os mecanismos de resistência em *A. baumannii*, envolvendo a pesquisa de elementos móveis, como as sequência de inserção (*ISAba1)* que estão associadas aos genes de oxacilinases.

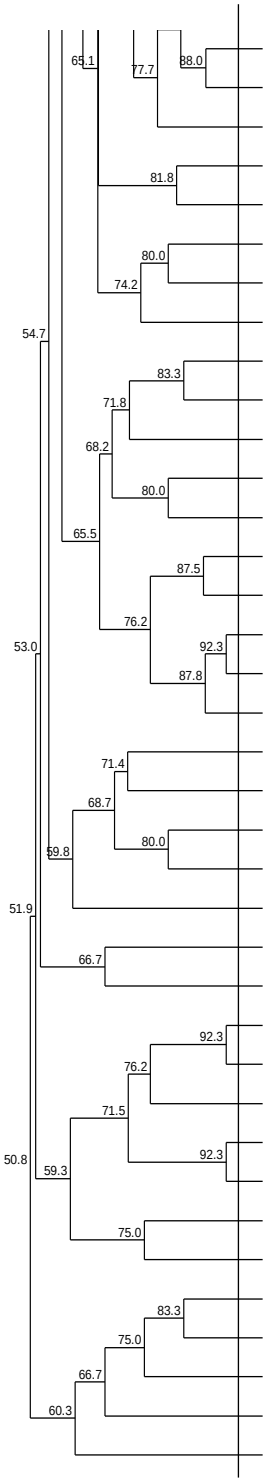
- Com os altos CIM de cefalosporinas encontrados no trabalho, identificar se a presença de β-lactamases cromossômicas do tipo AmpC ou outros mecanismos como bombas de efluxo e perda de porina estão relacionados com a resistência entre as amostras.

- Avaliar o vínculo epidemiológico dos *clusters* detectados por mais de um período em instituições diferentes, para melhor entendimento da disseminação dos isolados. Para que desta forma, possa estabelecer medidas eficientes que diminuam a emergência e a expansão de clones resistentes.

# APÊNDICE

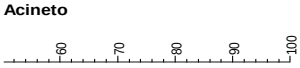



| Isolate | Date | Initial | | Origin/ Site | IPM | MEM | FEP | CAZ | CRO | PMB | OXA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HU UEL 4605 | 11-08-2004 | M.P.S | A | Tracheal secretion | 16 | 16 | 64 | >256 | >512 | 1 | 51,23 |
| Sc Ac 43 | 03-13-2010 | H.R | B | Hemoculture | 16 | 16 | 128 | >256 | >512 | 1 | 51 |
| Sc Ac 73 | 06-28-2010 | M.L.Z.F | B | Oral swabs | 32 | 32 | 128 | >256 | >512 | 2 | 51 |
| HUM KPC 215 | 02-07-2013 | I.R | C | Rectal swabs | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| Sc Ac 47 | 03-25-2010 | N.S | B | Tracheal secretion | 1 | 1 | 4 | 4 | 16 | 1 | 51 |
| Sc Ac 49 | 04-07-2010 | M.C | B | Infusion pump | 16 | 16 | 128 | 128 | >512 | 0.5 | 51,23 |
| HU UEL 4345 | 08-27-2007 | C.A.C | A | Tracheal secretion | 32 | 32 | 256 | >256 | 512 | 1 | 51,23 |
| Sc Ac 102 | 11-10-2010 | D.R.V | B | Urine | 64 | 64 | 128 | 8 | 64 | 2 | 51,23 |
| Sc Ac 46 | 03-19-2010 | M.S | B | Tracheal secretion | 4 | 8 | 128 | >256 | >512 | 1 | 51 |
| Sc Ab 7 | 05-17-2009 | I.W | B | Urine | 8 | 8 | 64 | 64 | 512 | 1 | 51,23 |
| Sc4 Ac21 | 05- 01-2014 | A.A.S | B | Liquor | 64 | 32 | 256 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac49 | 01-16-2012 | J.A.S.F | C | Tracheal secretion | 2 | 4 | 128 | 128 | >512 | 2 | 51 |
| HUM Ac85 | 01-10-2013 | A.M.S | C | Abdominal fluid | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM Ac45 | 12-16-2011 | J.A.S | C | Catheter tip | 32 | 32 | 32 | >256 | 64 | 2 | 51,23 |
| HUM Ac43 | 11-13-2011 | R.A.S.B | C | Hemoculture | 32 | 16 | 128 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac84 | 12-18-2012 | E.C | C | Urine | 32 | 16 | >256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 6 | 08-30-2011 | M.P.S.B | C | Rectal swabs | 16 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac57 | 02-28-2012 | M.A.L | C | Hemoculture | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 521 | 04-07-2014 | J.M.A | C | Rectal swabs | 64 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| HUM Ac127 | 05-13-2014 | A.F.O | C | Tracheal secretion | 32 | 32 | >256 | >256 | 512 | 0.25 | 51,23 |
| Sc Ac 26 | 01-22-2010 | G.N.F | B | Oral swab | 1 | 0.5 | >256 | >256 | >512 | 0.5 | 51 |
| HUM KPC 61 | 03-14-2012 | G.P.R | C | Rectal swabs | 16 | 16 | 256 | 256 | >512 | 2 | 51,23 |
| HUM KPC 67 | 04-02-2012 | R.B.E | C | Rectal swab | 64 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| Sc Ac 6 | 08-09-2009 | S.F | B | Nasal swab | 8 | 1 | >256 | >256 | >512 | 1 | 51,23 |
| HUM Ac72 | 07-22-2012 | L.L.A | C | Tracheal secretion | 16 | 16 | 32 | >256 | >512 | 2 | 51 |
| Sc Ab 34 | 06-22-2009 | M.J.S.D | B | Hemoculture | 0.5 | 2 | 128 | >256 | >512 | 1 | 51,23 |
| HUM Ac120 | 03-25-2014 | M.S.L | C | Catheter | 32 | 64 | >256 | >256 | 512 | 2 | 51,23 |
| HUM Ac13 | 04-25-2011 | J.A.B | C | Liquid peritoneal | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM KPC445 | 01-27-2014 | A.O.B | C | Rectal swab | 32 | 64 | 64 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac21 | 06-19-2011 | V.A.R | C | Tracheal secretion | 8 | 8 | >256 | >256 | >512 | 2 | 51 |
| HU UEL 5980 | 12-03-2007 | M.J.B | A | Secretion | 64 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| HUM Ac88 | 12-28-2012 | V.G | C | Hemoculture | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM Ac83 | 12-04-2012 | L.R.A.S.J | C | Tracheal secretion | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| Sc Ac 19 | 11-19-2009 | R.M.P.S | B | Tracheal secretion | 16 | 16 | 128 | >256 | >512 | 1 | 51 |
| HUM KPC 146 | 08-21-2012 | J.C.R | C | Rectal swabs | 16 | 32 | 128 | 128 | >512 | 2 | 51,23 |
| HUM Ac117 | 02-12-2014 | J.Q.M | C | Tracheal secretion | 32 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| HUM KPC 572 | 05-20-2014 | H.F.S | C | Rectal swabs | 64 | 64 | 64 | 256 | 512 | 0.25 | 51,23 |
| Sc Ab 29 | 06-04-2009 | P.F | B | Tracheal secretion | 8 | 8 | 256 | >256 | >512 | 1 | 51,23 |
| HUM Ac17 | 05-28-2011 | V.F.S | C | Hemoculture | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac121 | 03-24-2014 | S.A.P | C | Tracheal secretion | 16 | 16 | >256 | 256 | 512 | 0.25 | 51 |
| HUM Ac27 | 07-21-2011 | M.A.G | C | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| Sc4 Ac55 | 06-16-2014 | R.D.R | B | Hemoculture | 8 | 8 | 256 | 256 | >512 | 1 | 51,23 |
| Sc Ac 63 | 05-03-2010 | G.M.P | B | Rectal swabs | 64 | 64 | 128 | 16 | 32 | 2 | 51,23 |
| Sc4 Ac7 | 04-09-2014 | J.M | B | Nasal swabs | 64 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| Sc Ac 64 | 05-21-2010 | R.B.S.B | B | Ora/ Nasal swabs | 2 | 2 | >256 | >256 | >512 | 0.25 | 51 |
| Sc4 Ac113 | 11-10-2014 | G.S.J | B | Nasal swabs | 64 | 32 | >256 | 256 | >512 | 1 | 51,23 |
| Sc4 Ac123 | 12-17-2014 | L.U.R.C | B | Oral swabs | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac125 | 12-23-2014 | L.B.P | B | Oral swabs | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac58 | 06-22-2014 | A.F.S | B | Urine | 64 | 32 | 128 | 256 | >512 | 0.25 | 51,23 |
| HU UEL 570 | 09-03-2011 | L.P.L | A | Liquid abdominal cavity | 64 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HU UEL 18T | 06-20-1994 | - | A | Catheter tip | 1 | 2 | 128 | 64 | >512 | 2 | 51 |
| Sc4 Ac57 | 06-21-2014 | F.R.M.S | B | Catheter tip | 32 | 32 | >256 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac15 | 05-23-2011 | I.F | C | Tracheal secretion | 32 | 16 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac35 | 10-04-2011 | G.C | C | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac05 | 01-24-2011 | A.S.S | C | Catheter bridge | 1 | 2 | >256 | >256 | >512 | 2 | 51 |
| Sc Ab 4 | 05-12-2009 | J.T.S | B | Tracheal secretion | 1 | 2 | 64 | 128 | >512 | 1 | 51,23 |

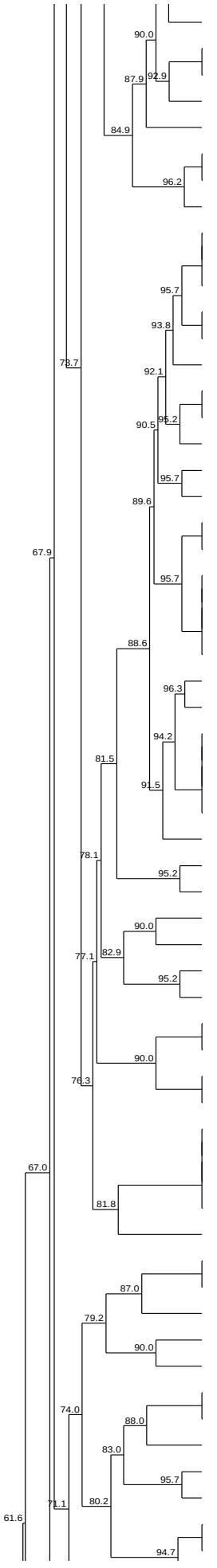



| Isolates | Date | Initial | | Origin/ Site | IPM | MEM | FEP | CAZ | CRO | PMB | OXA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HUM Ac05 | 01-24-2011 | A.S.S | C | Catheter bridge | 1 | 2 | >256 | >256 | >512 | 2 | 51 |
| Sc Ab 4 | 05-12-2009 | J.T.S | B | Tracheal secretion | 1 | 2 | 64 | 128 | >512 | 1 | 51,23 |
| HU UEL 102 | 10-21-1996 | C.V | A | Tracheal secretion | 1 | 2 | 256 | 256 | >512 | 2 | 51 |
| HUM KPC 11 | 09-14-2011 | A.Z.S | C | Rectal swabs | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| Sc Ac 20 | 11-02-2009 | M.R.S | B | Tracheal secretion | >64 | 32 | 128 | >256 | >512 | 0.25 | 51,23 |
| HU UEL 3813 | 10-11-2004 | Air circuit | A | Environment | 32 | 32 | 64 | >256 | >512 | 2 | 51 |
| HUM KPC 35 | 24-11-2011 | JCS | C | Swab retal | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| HUM Ac81 | 11-22-2012 | B.G | C | Hemoculture | 1 | 8 | >256 | >256 | >512 | 2 | 51 |
| Sc Ac 25 | 12-29-2009 | A.A | B | Oral swab | 8 | 0.5 | 16 | 16 | >512 | 2 | 51 |
| Sc Ac 58 | 04-10-2010 | S.R.S | B | Tracheal secretion | 16 | 16 | 128 | >256 | 512 | 1 | 51,23 |
| Sc Ac 59 | 04-14-2010 | O.G | B | Tracheal secretion | 16 | 16 | 128 | >256 | >512 | 1 | 51,23 |
| Sc Ac 76 | 07-06-2010 | J.B.M | B | Oral/ Nasal swabs | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| Sc Ac 86 | 09-16-2010 | F.P.B | B | Urine | 64 | 64 | 128 | 256 | >512 | 2 | 51,23 |
| HUM Ac 102 | 07-03-2013 | A.R.A | C | Rectal swabs | 1 | 2 | >256 | >256 | >512 | 2 | 51 |
| HUM KPC 341 | 08-28-2012 | N.M.C.S | C | Rectal swab | 32 | 32 | 256 | >256 | >512 | 2 | 51 |
| HUM KPC 260 | 04-20-2013 | D.L.S | C | Rectal swabs | 0.5 | 1 | 64 | >256 | >512 | 2 | 51 |
| HUM KPC 346 | 10-15-2013 | F.C.M | C | Rectal swabs | 32 | 32 | 256 | 256 | 512 | 2 | 51,23 |
| HUM KPC 86 | 04-20-2012 | A.M.A | C | Rectal swabs | 16 | 16 | >256 | >256 | >512 | 2 | 51,23 |
| HU UEL 24 | 11-27-1994 | L.T.S | A | Catheter tip | 2 | 1 | 16 | 32 | >512 | 2 | 51 |
| HUM Ac74 | 08-14-2012 | V.C | C | Tracheal secretion | 64 | 16 | 128 | 256 | >512 | 0.5 | 51,23 |
| HU UEL 6056 | 12-11-2007 | Air circuit | A | Environment | 32 | 32 | 64 | 256 | 512 | 2 | 51,23 |
| HUM KPC 273 | 04-29-2013 | J.A.M | C | Rectal swabs | 16 | 32 | >256 | 64 | >512 | 2 | 51,23 |
| HUM KPC 390 | 11-06-2013 | M.L.D.C | C | Rectal swabs | 16 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HU UEL 4071 | 10-27-2004 | A.A.S | A | Tracheal secretion | 1 | 2 | 64 | 64 | >512 | 2 | 51 |
| Sc Ac 60 | 04-18-2010 | J.R.S | B | Oral/Nasal swabs | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| HUM Ac 104 | 06-19-2013 | J.M.T | C | Catheter tip | 32 | 32 | 128 | >256 | 64 | 2 | 51,23 |
| HUM KPC 315 | 07-08-2013 | G.M.S.F | C | Rectal swabs | 32 | 32 | 128 | >256 | 64 | 2 | 51,23 |
| HUM KPC 227 | 03-02-2013 | B.B | C | Rectal swabs | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 113 | 05-24-2012 | G.M.S | C | Rectal swabs | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 264 | 04-15-2013 | D.J.K | C | Rectal swabs | 32 | 32 | 64 | >256 | >512 | 2 | 51,23 |
| Sc Ac 36 | 03-04-2010 | R.F.C | B | Tracheal secretion | 16 | 16 | 128 | >256 | >512 | 2 | 51 |
| Sc Ac 39 | 03-22-2010 | M.L.M | B | Oral/ Nasal swabs | 1 | 1 | 4 | 4 | 16 | 1 | 51,23 |
| HU UEL 14 | 10-23-1995 | Bed | A | Environment | 2 | 2 | 32 | 32 | >512 | 2 | 51 |
| HU UEL 18 | 11-07-1995 | Air ciruit | A | Environment | 1 | 1 | 16 | 32 | 512 | 2 | 51 |
| HU UEL 97 | 11-14-1995 | I.C | A | Enteral solution | 1 | 2 | 64 | 32 | 32 | 2 | 51 |
| HUM Ac28 | 07-25-2011 | O.V.M | C | Tracheal secretion | 0.5 | 1 | 8 | 16 | >512 | 2 | 51 |
| HUM KPC 74 | 04-02-2012 | L.S.C | C | Rectal swabs | 32 | 32 | 128 | >256 | >512 | 0.25 | 51,23 |

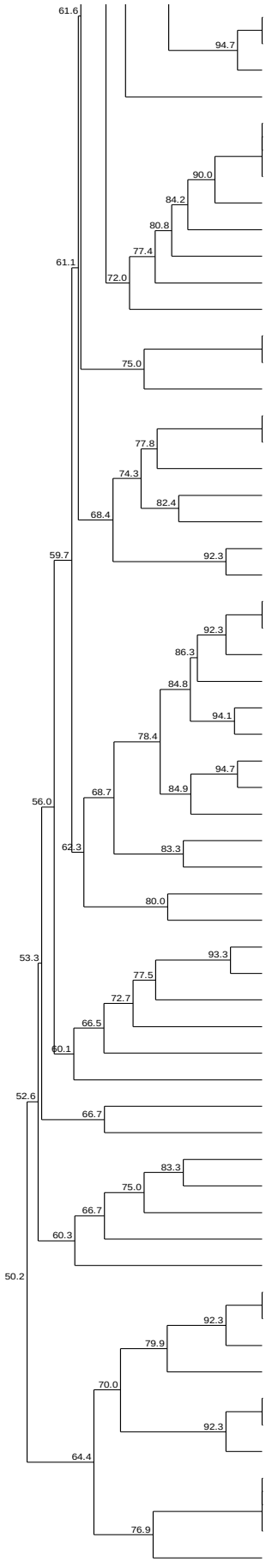
Dendograma obtido pelo "*Enterobacterial repetitive intergenic consensus" – Polymerase Chain Reaction* (ERIC-PCR) dos 93 *clusters* encontrado entre as 176 amostras de *A. baumannii* isoladas nos últimos vinte anos de três centros médicos do Norte do Paraná. Escala representando percentual de similaridade. A linha no dendrograma denota o limiar de 93% de homologia para a definição de grupos de similaridade genética. Concentração inibitória mínima – CIM (miligramas/ litro): IPM imipenem, MEM meropenem, FEP cefepima, CAZ ceftazidima, CRO cefriaxona, PMB polimixina B; OXA oxacilinase.




| Isolate | Date | Initial | | Origin/ Site | IPM | MEM | FEP | CAZ | CRO | PMB | OXA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HUM KPC 665 | 08-01-2014 | M.R.G.S. | C | Rectal swabs | 32 | 32 | 128 | >256 | >512 | 0.25 | 51,23 |
| Sc Ac 17 | 11-05-2009 | L.N.P | B | Urine | 8 | 16 | 256 | >256 | >512 | 1 | 51,23 |
| Sc Ac 20 | 11-02-2009 | M.R.S | B | Tracheal secretion | >64 | 32 | 128 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac 21 | 06-19-2011 | V.A.R | C | Tracheal secretion | 8 | 8 | >256 | >256 | >512 | 2 | 51 |
| Sc4 Ac32 | 05-06-2014 | G.P.Z | B | Swab anal | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac34 | 05-06-2014 | C.J.R | B | Swab anal | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac7 | 04-09-2014 | J.M | B | Nasal swabs | 64 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 ac49 | 05-25-2014 | E.P.P | B | Tracheal secretion | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac31 | 05-06-2014 | G.N.O | B | Swab Nasal e Oral | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac48 | 08-29-2014 | L.F.G | B | Sec. traqueal | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac23 | 04-29-2014 | B.B. | B | Hemoculture | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac51 | 06-08-2014 | F.L.F | B | Aspirado de pulmão | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| HUM Ac47 | 01-16-2012 | C.P. | C | Tracheal secretion | 32 | 32 | 64 | >256 | >512 | 2 | 51,23 |
| HUM KPC 35 | 24-11-2011 | JCS | C | Swab retal | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| HU UEL 3813 | 10-11-2004 | Air circuit | A | Environment | 32 | 32 | 64 | >256 | >512 | 2 | 51 |
| HUM Ac 72 | 07-22-2012 | L.L.A | C | Tracheal secretion | 16 | 16 | 32 | >256 | >512 | 2 | 51 |
| Sc4 Ac9 | 04-08-2014 | H.A.A | B | Anal swab | 32 | 32 | >256 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac54 | 06-16-2014 | R.A.B | B | Anal swab | 32 | 32 | >256 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac55 | 06-16-2014 | R.D.R | B | Hemoculture | 8 | 8 | 256 | 256 | >512 | 1 | 51,23 |
| HUM Ac 27 | 07-21-2011 | M.A.G | C | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM KPC 63 | 04-02-2012 | M.J.S | C | Rectal swab | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac 17 | 05-28-2011 | V.F.S | C | Hemoculture | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| Sc Ac 63 | 05-03-2010 | G.M.P | B | Rectal swabs | 64 | 64 | 128 | 16 | 32 | 2 | 51,23 |
| HUM Ac 80 | 11-22-2012 | B.G | C | Hemoculture | 4 | 4 | >256 | >256 | >512 | 0.5 | 51 |
| HUM Ac75 | 08-27-2012 | J.C.R | C | Líquid abdominal cavi. | 32 | 32 | >256 | >256 | >512 | 1 | 51,23 |
| HUM Ac 85 | 01-10-2013 | A.M.S | C | Abdominal fluid | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| Sc Ac 6 | 08-09-2009 | S.F | B | Nasal swab | 8 | 1 | >256 | >256 | >512 | 1 | 51,23 |
| Sc Ac 2 | 09-04-2009 | W.F.B | B | Tracheal secretion | 1 | 1 | >16 | >32 | >32 | 1 | 51,23 |
| Sc Ac 3 | 08-29-2009 | J.P | B | Tracheal secretion | 4 | 4 | >32 | >32 | >32 | 1 | 51,23 |
| Sc Ac 4 | 09-08-2009 | V.L | B | Tracheal secretion | 2 | 1 | >16 | >16 | >16 | 1 | 51 |
| Sc Ab 33 | 05-20-2009 | L.T | B | Tracheal secretion | 0.5 | 2 | >16 | >32 | >32 | 1 | 51,23 |
| Sc Ab 34 | 06-22-2009 | M.J.S.D | B | Hemoculture | 0.5 | 2 | 128 | >256 | >512 | 1 | 51,23 |
| Sc Ab 35 | 05-21-2009 | P.B | B | Tracheal secretion | 1 | 2 | >16 | >16 | >32 | 1 | 51,23 |
| Sc Ac 8 | 09-22-2009 | G.R.S | B | Urine | 2 | 1 | >16 | >32 | >32 | 1 | 51,23 |
| HUM KPC 67 | 04-02-2012 | R.B.E | | Rectal swabs | 64 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| HU UEL 530 | 12-08-2011 | M.H.S | A | Catheter tip | 8 | 8 | 256 | 128 | >512 | | 51,23 |
| HU UEL 570 | 09-03-2011 | L.P.L | A | Liquid abdominal cavi. | 64 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HU UEL 576 | 10-09-2011 | D.B.R | A | Tracheal secretion | 8 | 8 | 256 | >256 | >512 | 32 | 51,23 |
| Sc Ab 29 | 06-04-2009 | P.F | B | Tracheal secretion | 8 | 8 | 256 | >256 | >512 | 1 | 51,23 |
| Sc Ab 30 | 07-08-2009 | M.A.B | B | Tracheal secretion | 8 | 8 | 256 | >256 | >512 | 0.5 | 51,23 |
| HUM Ac 121 | 03-24-2014 | S.A.P | C | Tracheal secretion | 16 | 16 | >256 | 256 | 512 | 0.25 | 51 |
| HUM KPC 458 | 02-10-2014 | M.J | C | Rectal swab | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 |
| HUM KPC492 | 03-10-2014 | C.G | C | Rectal swab | 32 | 32 | 128 | 256 | 256 | 1 | 51,23 |
| HUM KPC482 | 03-10-2014 | M.F.S.L | C | Rectal swab | 32 | 32 | 128 | 256 | 256 | 1 | 51,23 |
| HUM Ac124 | 04-07-2014 | I.A.M | C | Tracheal secretion | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 |
| HUM KPC 445 | 01-27-2014 | A.O.B | C | Rectal swab | 32 | 64 | 64 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac 117 | 02-12-2014 | J.Q.M | C | Tracheal secretion | 32 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| HUM KPC 572 | 05-20-2014 | H.F.S | C | Rectal swabs | 64 | 64 | 64 | 256 | 512 | 0.25 | 51,23 |
| HU UEL 5980 | 12-03-2007 | M.J.B | A | Secretion | 64 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| HUM Ac 88 | 12-28-2012 | V.G | C | Hemoculture | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM Ac 83 | 12-04-2012 | L.R.A.S.J | C | Tracheal secretion | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM Ac 135 | 07-09-2014 | M.Z.S | C | Hemoculture | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| HUM Ac127 | 05-13-2014 | A.F.O | C | Tracheal secretion | 32 | 32 | >256 | >256 | 512 | 0.25 | 51,23 |
| HUM Ac137 | 07-07-2014 | J.Q.M | C | Tracheal secretion | >8 | >8 | 16 | >16 | >32 | S | 51,23 |
| HUM Ac 57 | 02-28-2012 | M.A.L | C | Hemoculture | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM Ac 129 | 05-19-2014 | H.R | C | Tracheacl secretion | >8 | >8 | >16 | >32 | >32 | S | 51.23 |



| Isolate | Date | Initial | | Origin/ Site | IPM | MEM | FEP | CAZ | CRO | PMB | OXA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HUM Ac 57 | 02-28-2012 | M.A.L | C | Hemoculture | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| HUM Ac 129 | 05-19-2014 | H.R | C | Tracheacl secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| HUM Ac131 | 05-19-2014 | M.A.M | | Catheter | | | | | | | |
| HUM Ac 120 | 03-25-2014 | M.S.L | C | Catheter bridge | 32 | 64 | >256 | >256 | 512 | 2 | 51,23 |
| HUM Ac 13 | 04-25-2011 | J.A.B | C | Liquid peritoneal | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac125 | 05-20-2014 | F.H.G | C | Tracheal secretion | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| HUM KPC 521 | 04-07-2014 | J.M.A | C | Rectal swabs | 64 | 64 | 128 | >256 | >512 | 1 | 51,23 |
| HUM KPC 559 | 04-24-2014 | E.S | C | Rectal swabs | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac69 | 07-24-2014 | K.C.M | B | Urine | >8 | >8 | >16 | >16 | >32 | S | 51,23 |
| Sc4 Ac74b | 08-25-2014 | J.H.N | B | Anal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac97 | 09-22-2014 | A.C.B | B | Anal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac58 | 06-22-2014 | A.F.S | B | Urine | 64 | 32 | 128 | 256 | >512 | 0.25 | 51,23 |
| Sc4 Ac71 | 07-25-2014 | A.B | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac104 | 10-03-2014 | M.R.B.M | B | Hemoculture | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac103 | 10-03-2014 | R.V.M | B | Oral/ Nasal swabs | 32 | 32 | >256 | 256 | >512 | 1 | 51,23 |
| Sc4 Ac83 | 08-28-2014 | C.J | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac108 | 10-13-2014 | P.F | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac107 | 10-06-2014 | A.R | B | Anal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac122 | 12-17-2014 | O.S | B | Anal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac77 | 08-12-2014 | J.A.P | B | Anal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac91 | 09-08-2014 | R.A.S | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac109 | 10-14-2014 | J.P | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac119 | 12-12-2014 | P.C.P | B | Oral swab | >8 | >8 | >32 | >32 | >32 | S | 51,23 |
| Sc4 Ac75 | 07-23-2014 | I.L.V.P | B | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac92 | 09-09-2014 | H.M.P | B | Anal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac125 | 12-23-2014 | L.B.P | B | Oral swabs | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac128 | 12-29-2014 | J.C.P | B | Wound secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac101 | 09-29-2014 | J.R.F | B | Oral/Nasal swab | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac111 | 10-17-2014 | F.C.V | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac118 | 12-11-2014 | A.R.M.B | B | Tracheal secretion | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac120 | 12-12-2014 | H.S.M | B | Catheter tip | >8 | >8 | >16 | >32 | >32 | S | 51,23 |
| Sc4 Ac123 | 12-17-2014 | L.U.R.C | B | Oral swabs | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac113 | 11-10-2014 | G.S.J | B | Nasal swabs | 64 | 32 | >256 | 256 | >512 | 1 | 51,23 |
| Sc4 Ac124 | 12-19-2014 | M.D.R.F | B | Tracheal secretion | >8 | >8 | >32 | >32 | >32 | S | 51,23 |
| HU UEL 18T | 06-20-1994 | - | A | Catheter tip | 1 | 2 | 128 | 64 | >512 | 2 | 51 |
| Sc4 Ac57 | 06-21-2014 | F.R.M.S | B | Catheter tip | 32 | 32 | >256 | >256 | >512 | 0.25 | 51,23 |
| Sc Ac 64 | 05-21-2010 | R.B.S.B | B | Ora/ Nasal swabs | 2 | 2 | >256 | >256 | >512 | 0.25 | 51 |
| Sc4 Ac94 | 09-11-2014 | J.S.M | B | Tracheal secretion | 32 | 32 | 128 | 256 | >512 | 1 | 51,23 |
| Sc4 Ac76 | 08-07-2014 | D.L.S | B | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| Sc4 Ac81 | 08-22-2014 | M.C.S | B | Gastrostomy secreti. | >8 | >8 | >32 | >32 | >32 | S | 51,23 |
| HUM KPC 11 | 09-14-2011 | A.Z.S | C | Rectal swabs | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 36 | 12-13-2011 | G.C | C | Rectal swabs | 8 | >8 | >16 | >16 | >32 | - | 51,23 |
| Sc Ac 15 | 11-02-2009 | F.A.L | B | Tracheal secretion | 8 | 16 | >16 | >32 | >32 | 1 | 51 |
| Sc Ac 16 | 11-10-2009 | O.C | B | Tracheal secretion | 8 | 16 | >16 | >32 | >32 | 0.5 | 51,23 |
| Sc Ac 19 | 11-19-2009 | R.M.P.S | B | Tracheal secretion | 16 | 16 | 128 | >256 | >512 | 1 | 51 |
| Sc Ac 22 | 12-09-2009 | R.L.V | B | Urine | 16 | 8 | >16 | >16 | >32 | 1 | 51,23 |
| HU UEL 102 | 10-21-1996 | C.V | A | Tracheal secretion | 1 | 2 | 256 | 256 | >512 | 2 | 51 |
| HUM Ac 49 | 01-16-2012 | J.A.S.F | C | Tracheal secretion | 2 | 4 | 128 | 128 | >512 | 2 | 51 |
| HUM Ac50 | 01-18-2012 | C.S.L. | C | Tracheal secretion | 0.5 | 2 | >16 | >32 | >32 | S | 51 |
| HUM Ac 45 | 12-16-2011 | J.A.S | C | Catheter tip | 32 | 32 | 32 | >256 | 64 | 2 | 51,23 |
| HUM Ac 84 | 12-18-2012 | E.C | C | Urine | 32 | 16 | >256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 6 | 08-30-2011 | M.P.S.B | C | Rectal swabs | 16 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| Sc Ab 1 | 04-19-2009 | W.S.B | B | Tracheal secretion | 2 | 2 | >16 | >16 | >32 | 1 | 51 |
| Sc Ab 4 | 05-12-2009 | J.T.S | B | Tracheal secretion | 1 | 2 | 64 | 128 | >512 | 1 | 51,23 |
| HUM Ac 05 | 01-24-2011 | A.S.S | C | Catheter bridge | 1 | 2 | >256 | >256 | >512 | 2 | 51 |
| HUM Ac 43 | 11-13-2011 | R.A.S.B | C | Hemoculture | 32 | 16 | 128 | >256 | >512 | 0.25 | 51,23 |
| HUM Ac53 | 01-23-2012 | J.R.C.S | C | Tracheal secretion | 32 | 32 | 128 | >256 | >512 | 1 | 51,23 |
| HUM Ac 15 | 05-23-2011 | I.F | C | Tracheal secretion | 32 | 16 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac16 | 05- 30-2011 | M.B.F.P. | C | Catheter bridge | 64 | 32 | >16 | >16 | >32 | S | 51,23 |



| Isolate | Date | Initial | | Origin/ Site | IPM | MEM | FEP | CAZ | CRO | PMB | OXA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| HUM Ac 15 | 05-23-2011 | I.F | C | Tracheal secretion | 32 | 16 | 128 | >256 | >512 | 2 | 51,23 |
| HUM Ac16 | 05- 30-2011 | M.B.F.P. | C | Catheter bridge | 64 | 32 | >16 | >16 | >32 | S | 51,23 |
| HUM Ac34 | 09-24-2011 | S.L.S. | C | Tracheal secretion | 32 | 32 | >16 | >16 | >32 | S | 51,23 |
| HUM KPC 61 | 03-14-2012 | G.P.R | C | Rectal swabs | 16 | 16 | 256 | 256 | >512 | 2 | 51,23 |
| Sc Ac 49 | 04-07-2010 | M.C | B | Infusion pump | 16 | 16 | 128 | 128 | >512 | 0.5 | 51,23 |
| Sc Ac 50 | 04-07-2010 | D.A.S | B | Dispenser | 16 | 16 | >16 | 8 | 16 | 1 | 51 |
| Sc Ac 52 | 04-07-2010 | L.S.M | B | Heart monitor | 16 | 16 | >16 | 4 | 16 | 1 | 51 |
| Sc Ac 47 | 03-25-2010 | N.S | B | Tracheal secretion | 1 | 1 | 4 | 4 | 16 | 1 | 51 |
| HU UEL 4345 | 08-27-2007 | C.A.C | A | Tracheal secretion | 32 | 32 | 256 | >256 | 512 | 1 | 51,23 |
| Sc Ac 46 | 03-19-2010 | M.S | B | Tracheal secretion | 4 | 8 | 128 | >256 | >512 | 1 | 51 |
| Sc Ac 102 | 11-10-2010 | D.R.V | B | Urine | 64 | 64 | 128 | 8 | 64 | 2 | 51,23 |
| Sc Ac 59 | 04-14-2010 | O.G | B | Tracheal secretion | 16 | 16 | 128 | >256 | >512 | 1 | 51,23 |
| Sc Ac 34 | 03-06-2010 | L.S.M | B | Tracheal secretion | 32 | 16 | >16 | 4 | 16 | 0.5 | - |
| Sc Ac 36 | 03-04-2010 | R.F.C | B | Tracheal secretion | 16 | 16 | 128 | >256 | >512 | 2 | 51 |
| Sc Ac 39 | 03-22-2010 | M.L.M | B | Oral/ Nasal swabs | 1 | 1 | 4 | 4 | 16 | 1 | 51,23 |
| Sc Ab 7 | 05-17-2009 | I.W | B | Urine | 8 | 8 | 64 | 64 | 512 | 1 | 51,23 |
| Sc Ab 8 | 05-19-2009 | D.S | B | Nasal swabs | 8 | 8 | >16 | >16 | >32 | 0.5 | 51,23 |
| Sc Ac 73 | 06-28-2010 | M.L.Z.F | B | Oral swabs | 32 | 32 | 128 | >256 | >512 | 2 | 51 |
| HU UEL 4605 | 11-08-2004 | M.P.S | A | Tracheal secretion | 16 | 16 | 64 | >256 | >512 | 1 | 51,23 |
| Sc Ac 43 | 03-13-2010 | H.R | B | Hemoculture | 16 | 16 | 128 | >256 | >512 | 1 | 51 |
| HUM Ac 04 | 01-06-2011 | V.F.H.R | C | Urine | 32 | 32 | 128 | 256 | 256 | 0.25 | 51,23 |
| HUM KPC 215 | 02-07-2013 | I.R | C | Rectal swabs | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HUM KPC 118 | 06-30-2012 | O.C.D | C | Rectal swabs | 16 | 16 | >16 | >16 | >32 | S | 51,23 |
| HUM KPC 86 | 04-20-2012 | A.M.A | C | Rectal swabs | 16 | 16 | >256 | >256 | >512 | 2 | 51,23 |
| HUM KPC 346 | 10-15-2013 | F.C.M | C | Rectal swabs | 32 | 32 | 256 | 256 | 512 | 2 | 51,23 |
| HUM KPC 260 | 04-20-2013 | D.L.S | C | Rectal swabs | 0.5 | 1 | 64 | >256 | >512 | 2 | 51 |
| HUM KPC 314 | 07-09-2013 | M.A.S | C | Rectal swab | 16 | 16 | >256 | >256 | >512 | 1 | 51,23 |
| Sc Ac 26 | 01-22-2010 | G.N.F | B | Oral swab | 1 | 0.5 | >256 | >256 | >512 | 0.5 | 51 |
| HUM Ac 100 | 06-20-2013 | R.G.R | C | Rectal swab | 16 | 16 | 128 | 256 | 256 | 1 | 51,23 |
| HUM KPC 341 | 08-28-2012 | N.M.C.S | C | Rectal swabs | 32 | 32 | 256 | >256 | >512 | 2 | 51 |
| HUM Ac 102 | 07-03-2013 | A.R.A | C | Rectal swabs | 1 | 2 | >256 | >256 | >512 | 2 | 51 |
| Sc Ac 25 | 12-29-2009 | A.A | B | Oral swab | 8 | 0.5 | 16 | 16 | >512 | 2 | 51 |
| Sc Ac 58 | 04-10-2010 | S.R.S | B | Tracheal secretion | 16 | 16 | 128 | >256 | 512 | 1 | 51,23 |
| Sc Ac 76 | 07-06-2010 | J.B.M | B | Oral/ Nasal swabs | 32 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| Sc Ac 86 | 09-16-2010 | F.P.B | B | Urine | 64 | 64 | 128 | 256 | >512 | 2 | 51,23 |
| HUM Ac 110 | 09-11-2013 | T.K | C | Tracheal secretion | 0.5 | 2 | >16 | >16 | >32 | S | 51 |
| HUM KPC 273 | 04-29-2013 | J.A.M | C | Rectal swabs | 16 | 32 | >256 | 64 | >512 | 2 | 51,23 |
| HUM Ac 74 | 08-14-2012 | V.C | C | Tracheal secretion | 64 | 16 | 128 | 256 | >512 | 0.5 | 51,23 |
| HU UEL 6056 | 12-11-2007 | Air circuit | A | Environment | 32 | 32 | 64 | 256 | 512 | 2 | 51,23 |
| HU UEL 24 | 11-27-1994 | L.T.S | A | Catheter tip | 2 | 1 | 16 | 32 | >512 | 2 | 51 |
| HUM KPC 390 | 11-06-2013 | M.L.D.C | C | Rectal swabs | 16 | 32 | 128 | >256 | >512 | 2 | 51,23 |
| HU UEL 4071 | 10-27-2004 | A.A.S | A | Tracheal secretion | 1 | 2 | 64 | 64 | >512 | 2 | 51 |
| Sc Ac 60 | 04-18-2010 | J.R.S | B | Oral/Nasal swabs | 32 | 32 | >256 | >256 | >512 | 2 | 51,23 |
| HU UEL 14 | 10-23-1995 | Bed | A | Environment | 2 | 2 | 32 | 32 | >512 | 2 | 51 |
| HU UEL 18 | 11-07-1995 | Air ciruit | A | Environment | 1 | 1 | 16 | 32 | 512 | 2 | 51 |
| HU UEL 97 | 11-14-1995 | I.C | A | Enteral solution | 1 | 2 | 64 | 32 | 32 | 2 | 51 |
| HUM Ac 28 | 07-25-2011 | O.V.M | C | Tracheal secretion | 0.5 | 1 | 8 | 16 | >512 | 2 | 51 |
| HUM KPC 74 | 04-02-2012 | L.S.C | C | Rectal swabs | 32 | 32 | 128 | >256 | >512 | 0.25 | 51,23 |
| HUM KPC 264 | 04-15-2013 | D.J.K | C | Rectal swabs | 32 | 32 | 64 | >256 | >512 | 2 | 51,23 |
| HUM KPC 421 | 12-17-2013 | K.D.F | C | Rectal swabs | 16 | 16 | >16 | >32 | >32 | S | 51,23 |
| HUM KPC 113 | 05-24-2012 | G.M.S | C | Rectal swabs | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |
| Sc4 Ac21 | 05- 01-2014 | A.A.S | B | Liquor | 64 | 32 | 256 | >256 | >512 | 0.25 | 51,23 |
| HUM KPC 315 | 07-08-2013 | G.M.S.F | C | Rectal swabs | 32 | 32 | 128 | >256 | 64 | 2 | 51,23 |
| HUM KPC 325 | 08-01-2013 | R.S.O | C | Rectal swabs | 16 | 16 | >16 | >16 | >32 | S | 51,23 |
| HUM Ac 104 | 06-19-2013 | J.M.T | C | Catheter bridge | 32 | 32 | 128 | >256 | 64 | 2 | 51,23 |
| HUM Ac 112 | 11-11-2013 | S.R.S | C | Hemoculture | 32 | 32 | 128 | 256 | 512 | 1 | 51,23 |
| HUM KPC 284 | 05-29-2013 | P.A.M | C | Rectal swabs | 16 | 16 | >16 | >16 | >32 | S | 51,23 |
| HUM KPC 338 | 08-27-2013 | O.M | C | Rectal swabs | 16 | 16 | >16 | >16 | >32 | S | 51,23 |
| HUM KPC 227 | 03-02-2013 | B.B | C | Rectal swabs | 32 | 32 | 256 | >256 | >512 | 2 | 51,23 |

Dendograma obtido pelo "*Enterobacterial repetitive intergenic consensus" – Polymerase Chain Reaction* (ERIC-PCR) das 176 amostras de *A. baumannii* isoladas nos últimos vinte anos de três centros médicos do Norte do Paraná. Escala representando percentual de similaridade.. Concentração inibitória mínima – CIM (miligramas/ litro): IPM imipenem, MEM meropenem, FEP cefepima, CAZ ceftazidima, CRO cefriaxona, PMB polimixina B; OXA oxacilinase.

# ANEXOS

# Normas para publicação no periódico: Journal of Hospital Infection (JHI)

## Guide for Authors

**About the Journal**

The *Journal of Hospital Infection* (JHI) is the editorially independent scientific publication of the Healthcare Infection Society (HIS). The aim of the Journal is to publish high quality research and information relating to infection prevention and control that is relevant to an international audience.

**Scope of the Journal**

JHI welcomes submissions that relate to all aspects of infection prevention and control in healthcare settings. This includes submissions that:
provide new insight into the epidemiology, surveillance, or prevention and control of healthcare-associated infections and antimicrobial resistance in healthcare settings;

- provide new insight into cleaning, disinfection and decontamination;
- provide new insight into the design of healthcare premises;
- describe novel aspects of outbreaks of infection;
- throw light on techniques for effective antimicrobial stewardship;
- describe novel techniques (laboratory-based or point of care) for the detection of infection or antimicrobial resistance in the healthcare setting, particularly if these can be used to facilitate infection prevention and control;
- improve understanding of the motivations of safe healthcare behaviour, or describe techniques for achieving behavioural and cultural change;
- improve understanding of the use of IT systems in infection surveillance and prevention and control.

We also welcome submissions that relate to national policies or guidelines, especially where the subject matter is of international relevance.

Although our readership is predominantly clinical, we are also pleased to received basic science submissions that have clinical relevance.

**Article types**

The Journal invites articles of the following types:

**Full length, original research articles**
This is the usual format for publishing original research.
The word limit is 4000 words of text, which includes the structured summary of up to 250 words, text, acknowledgements and references. Each figure and/or table counts as 200 words towards the total. JHI accepts electronic supplementary material to support and enhance your scientific research.
**Short reports**
This format is ideal for reporting smaller original research studies.
The format is the same as for a full length, original research article, except that the summary of up to 100 words should be unstructured.
The word limit is 2000 words of text, with no more than two figures or tables and a maximum of ten references.
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We welcome general reviews that summarize the current understanding and research on a topic, and systematic reviews that provide a thorough critical assessment of current evidence.
Authors of systematic reviews and meta-analyses are encouraged to present these according to the PRISMA guidelines for systematic reviews and meta-analyses (www.prisma-statement.org/). Before you start work on a review we do advise that you check with our office that the topic of your review is suitable and that there are no similar reviews in progress.
For general reviews, an unstructured summary of up to 250 words is required; for systematic reviews, please provide a structured summary of up to 250 words.
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**Commentaries**
Commentaries are by invitation only. These are intended to provide background and context for published

articles, and are usually written by an editor or referee. The word limit is 700 words, and a maximum of 10 references. No summary, tables or figures are allowed.

**Editorials**
Editorials are by invitation only. These provide a broad overview of topics that are relevant to infection prevention and control, but are less detailed than a review article. Word and reference limits will be agreed with the Editor at the time of invitation. Readers are welcome to submit suggestions for editorial subject matter to our office.
**Opinions**
Opinions are by invitation only. These provide the authors viewpoint on widespread concepts and methods. Authors can comment on the strengths and weaknesses of an approach in a constructive and evidence based form. The word limit is 700 words and a maximum of 10 references. No summary, tables or figures are allowed. Readers are welcome to suggest subject matter for opinions to our office.
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**Outbreak reports**
We welcome these, but there should be something new about them, e.g. caused by a novel organism, associated with a new source or identified or controlled using novel methods

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The JHI requires a hand signed signature from every individual author listed confirming that they have read and agree to the final draft before submission. Signatures created on a computer cannot be accepted.

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All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

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