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EPIDEMIOLOGIA MOLECULAR DE *Acinetobacter baumannii* RESISTENTES A CARBAPENÊMICOS

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Dissertação apresentada à Faculdade de Ciências da Saúde da Universidade Federal da Grande Dourados para obtenção do título de Mestre em Ciências da Saúde

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Orientadora: Profa. Dra. Simone Simionatto

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Dedico este trabalho...

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“Não existe nada de completamente errado no mundo, mesmo um relógio parado, consegue estar certo duas vezes por dia.”

Paulo Coelho

Sumário

Lista de abreviaturas e siglas

AAC - *Aminoglycoside acetyltransferases*

AB - Acinetobacter baumannii

A. baumannii - Acinetobacter baumannii

A. baylyi - Acinetobacter baylyi

ABC - ATP *binding cassette*

AbeM: *Acinetobacter baumannii efflux pump of* MATE *family*

AbeS: *Acinetobacter baumannii efflux pump of* SMR *family*

A. calcoaceticus - Acinetobacter calcoaceticus

ADCs - *Acinetobacter-derived cefalosporinases*

Ade: *Acinetobacter baumannii multidrug-resistant efflux pump*

A. junii – Acinetobacter junii

AMES - *Aminoglycoside-modifying enzymes*

AmpC - *Cephalosporinase chromosomal*

AmvA: *Acinetobacter baumannii methyl viologen and antimicrobial resistance protein*

A. nosocomialis - Acinetobacter nosocomialis

ANT: *Aminoglycoside adenyltransferases*

ANVISA - Agência Nacional de Vigilância Sanitária

Apa - *Acetobacter pasteurianus*

APH: *Aminoglycoside phosphotransferases*

A. pittii - Acinetobacter pittii

ArmA: Armillaria mellea

ATCC - *American Type Cuture Collection*

bla – Betalactamase

BGN – Bacilo Gram-Negativo

β-lactamase - Betalactamase

β-lactâmicos - Betalactâmicos

C - citosina

CarO: *Carbapenem-associated Outer Membrane Protein*

CC - *Clonal complex*

CDC - *Centers for Disease Control and Prevention*

CEP – Comitê de Ética em Pesquisa

CHDL - *Carbapenem-hydrolyzing class D β-lactamase*

CIM - Concentração Inibitória Mínima

CmlA: *Chloramphenicol resistance Acinetobacter*

CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico

COG - C*lusters* ou *Orthologous groups*

com – Cellular Outer Membrane

CraA: *Chloramphenicol resistance Acinetobacter*

CRAB - *Carbapenem-resistant Acinetobacter baumannii*

CSAB - *Carbapenem-sensitive Acinetobacter baumannii*

CTX-M - *Cefotaxime hydrolyzing capabilities*

CVC - Cateter venoso central

DMT - *Drug metabolite transporter*

DNA - *Deoxyribonucleic acid*

DPOC - Doença pulmonar obstrutiva crônica

ECDC – *European Centre for Disease Prevention and Control*

E. coli – Escherichia coli

ESβL - *Extended Spectrum β-lactamase*

et. al - e outros

FUNDECT/MS - Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul

G - Guanina

GES - *Guiana Extended Spectrum*

GIM - *German Imipenemase*

GyrA - DNA *Gyrase*

hs - horas

IMP - *Imipenemase*

INICC - *International Nosoconial Infection Control Consortium*

IRAS - Infecções relacionadas à assistência à saúde

IS - *Insertion Sequence*

IS*Aba - Insertion Sequence Acinetobacter baumannii*

ITU - Infecção do trato urinário

kb - kilo bases

kDa - kilodaltons

K. pneumoniae - Klebsiella pneumoniae

KPC - *Klebsiella pneumoniae carbapenemase*

LPS - Lipopolissacarídeo

lpx - lipoxygenase

MALDI-TOF MS - *Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry*

MATE - *Multidrug and toxic compound exporters*

MDR - *Multidrug resistance*

MDRAB - *Multidrug resistance Acinetobacter baumannii*

MFS - *Major superfamily facilitator*

MIC - *Minimum Inhibitory Concentration*

MLST - *Multilocus Sequence Typing*

MS - Mato Grosso do Sul

MβL - *Metallo β-lactamase*

NDM - *New Delhi Metallo-β-lactamase*

No - Número

OMP - *Outer Membrane Protein*

OMS - Organização Mundial da Saúde

OMV - *Outer Membrane Vesicles*

ORF - *Open Reading Frame*

OXA - *Oxacillinase*

P. aeruginosa – Pseudomonas aeruginosa

PAI - *Pathogenicity islands*

ParC - *Partitioning of the nucleoid partition*

PAVM – Pneumonia associada a ventilação mecânica

pb - pares de base

PBP - *Penicillin Binding Protein*

PCR - *Polimerase Chain Reaction*

PDR - *Pandrug resistance*

PER - *Pseudomonas Extended Resistant*

PFGE - *Pulsed Field Gel Electrophoresis*

pil - pillus

pmr - polymyxin resistance

P. mirabilis – Proteus mirabilis

RedCap - *Research Electronic Data Capture*

Rep-PCR - *Repetitive Element Palindromic - Polimerase Chain Reaction*

rmt - rRNA *methylase*

rRNA - RNA ribossomal

RNAt - RNA transportador

RND - *Resistance nodulation division*

SAS - *Statistical Analysis System*

SCOPE - *Surveillance and Control of Pathogens of Epidemiological Importance*

SENTRY - SENTRY *Antimicrobial Surveillance Program*

SHV - *Sulfhydryl-Variable β-lactamase*

SIM - *Seul Imipenemase*

SMR - *Small Multidrug Resistance*

SPM - São Paulo Metalo-β-lactamase

ST - *Sequence Type*

TEM - *Temoniera β-lactamase*

TetA: *Tetracycline resistant Acinetobacter*

Tn - Transposon

UTI - Unidade de Terapia Intensiva

VEB - *Vietnam Extended-Spectrum β-lactamase*

VIM - *Verona Imipenemase*

VM - Ventilação mecânica

WHO – *World Health Organization*

30S - Subunidade ribossomal 30

Lista de anexos

Lista de figuras

Lista de tabelas

Resumo

Maciel, W. G. Epidemiologia molecular de *Acinetobacter baumannii* resistentes a carbapenêmicos. Dourados/MS: Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados; 2016. 139 p.

Introdução: *Acinetobacter baumannii* são responsáveis por infecções hospitalares e altas taxas de morbidade e mortalidade de pacientes hospitalizados. **Objetivo:** Avaliar o perfil epidemiológico e molecular de cepas de *A. baumannii* resistentes a carbapenêmicos isoladas nas Unidades de Terapia Intensiva (UTIs) Neonatal e Adulto de um hospital público de Dourados/Mato Grosso do Sul (MS), visando identificar os fatores de risco relacionados à infecção e colonização ocasionadas por este microrganismo. **Métodos:** A identificação bacteriana foi realizada pelo sistema automatizado Vitek®2 e confirmada por *Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry* (MALDI-TOF MS) e o perfil de sensibilidade aos antibióticos foi avaliado através do Vitek®2. A presença de genes codificadores de β-lactamases foi avaliada pela Reação em Cadeia da Polimerase (PCR). O perfil clonal e a ancestralidade das cepas foram determinados por Eletroforese em Campo Elétrico Pulsado (PFGE) e *Multilocus Sequence Typing* (MLST), respectivamente. Estudos de caso-controle foram realizados a fim de identificar os fatores de risco envolvidos na aquisição de *A. baumannii* resistentes a carbapenêmicos nas UTIs Neonatal e Adulto. **Resultados:** Durante o período de setembro de 2013 a setembro de 2015 foram isoladas 59 cepas de *A. baumannii* resistentes a carbapenêmicos, sendo 18 isoladas de recém-nascidos e 41 isoladas de pacientes adultos. As cepas apresentaram sensibilidade apenas aos antibióticos amicacina, ampicilina-sulbactam, colistina, gentamicina e tigeciclina. Todos os isolados apresentaram a sequência de inserção IS*Aba1* à montante do gene $bla_{\text{OXA-23}}$ e o gene $bla_{\text{OXA-51}}$. As cepas de *A. baumannii* foram clonalmente relacionadas e pertenciam ao genótipo ST1, na sua maioria. Além disso, observou-se um surto ocasionado por *A. baumannii* resistentes a carbapenêmicos em recém-nascidos. Síndromes respiratórias, prematuridade, exposição prévia a antibióticos, uso de betalactâmicos, uso de cefalosporinas e acesso periférico foram considerados fatores associados à colonização por *A. baumannii* em recém-nascidos. Na UTI adulto, foi observado que 34,.6% (n=9) dos pacientes evoluíram ao óbito decorrente de sepse por *A. baumannii* pertencentes ao genótipo ST79. Uso de tubo nasogástrico, hemodiálise e uso de cefalosporinas foram considerados fatores de risco para infecção e colonização ocasionadas por *A. baumannii* resistentes a carbapenêmicos em pacientes adultos. **Conclusão:** Os

resultados obtidos indicam a disseminação de cepas de *A. baumannii* resistentes a carbapenêmicos em UTIs Neonatal e Adulto de um hospital público de Dourados/MS, destacando a necessidade do desenvolvimento de estratégias eficazes para o controle e prevenção de infecções hospitalares.

Palavras-chave: Resistência bacteriana, UTI Neonatal, UTI Adulto.

Abstract

Introduction: *Acinetobacter baumannii* are related to hospital infections, in addition are responsible for high rates of morbidity and mortality in hospitalized patients. **Aim:** This study evaluates the epidemiological and molecular profile carbapenem-resistant *A. baumannii* strains isolated in the neonatal and adult Intensive Care Unit of a public hospital in Dourados/MS and identify risk factors related to infection and colonization caused by this microorganism. **Methods:** Bacterial identification was performed by Vitek®2 automatized system and confirmed by Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) and antimicrobial susceptibilities was performed by Vitek®2 automatized system. The presence of β-lactamase genes was evaluated by Polymerase Chain Reaction (PCR). The profile clonal and genetic relationship were determined by Pulsed Field Gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST), respectively. Case-control studies were conducted to identify risk factors for acquisition of carbapenem- resistant *A. baumannii* in the neonatal and adult Intensive Care Unit (ICU). **Results:** During September/2013 to September/2015 59 *A. baumannii* strains were isolated, 18 strains of newborns and 41 isolated from adult patients. These strains showed sensitivity only to amikacin, ampicillin-sulbactam, colistin, gentamicin and tigecycline. The insertion sequence IS*Aba1* upstream $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes were identified in all strains. Carbapenem-resistant strains were clonally related and the predominant genotype identified was ST1. In addition, an outbreak carbapenem-resistant *A. baumannii* has been observed in newborns. Respiratory syndromes, prematurity, prior exposure to antibiotics, use of beta-lactams, cephalosporins and use of peripheral access were considered factors associated with the colonization of *A. baumannii* in newborns. In the adult ICU, 34.6% (n = 9) of the patients evolved to death due to A. baumannii sepsis belonging to the ST79 genotype. Use of the nasogastric tube, hemodialysis and use of cephalosporins were considered risk factors for the infection and colonization of carbapenem-resistant *A. baumannii* strains in adult patients. **Conclusion:** The results indicate the spread of carbapenem-resistant *A. baumannii* strains in the neonatal and adult Intensive Care Unit of a public hospital in Dourados/MS, highlighting the need to develop effective strategies for control and prevention of nosocomial infections.

Keywords: Bacterial resistance, Neonatal ICU, Adult ICU.

1. Introdução

As infecções hospitalares são responsáveis pelo aumento no tempo de internação, altas taxas de morbidade e mortalidade e altos custos assistenciais, caracterizando um importante problema de saúde pública. Além disso, proporcionam o risco de disseminação de microrganismos resistentes a antibióticos nos ambientes nosocomiais (DALTOÉ, BREIER, SANTOS, et al., 2014; BRASIL, 2015).

Acinetobacter spp. são considerados patógenos oportunistas na clínica médica, sendo reconhecidos por sua capacidade de acarretar infecções e colonizações (PELEG, SEIFERT, PATERSON, 2008; SCHIMITH BIER, LUIZ, SCHEFFER, et al., 2010; DOI, MURRAY, PELEG, 2015). Esse patógeno pertence à família *Moraxellaceae* (ROSSAU, VAN LANDSCHOOT, GILLIS, et al., 1991) e compreende 41 espécies diferentes (EUZEBY, 2016), sendo *Acinetobacter baumannii* a espécie mais importante clinicamente (PELEG, SEIFERT, PATERSON, 2008; MOLINA, CISNEROS, FERNANDEZ-CUENCA, et al., 2010; MARTINS, BARTH, 2013). São responsáveis por diferentes tipos de infecções, como pneumonias, meningites, infecções urinárias, respiratórias e de sítios cirúrgicos (de BREIJ, DIJKSHOORN, LAGENDIJK, et al., 2010; SCHIMITH BIER, LUIZ, SCHEFFER, et al., 2010; SHENG, LIAO, LAUDERDALE, et al., 2010), acometendo principalmente pacientes que foram submetidos a procedimentos invasivos, imunocomprometidos e internados em Unidades de Terapia Intensiva (UTIs) (MARTINS, BARTH, 2013; KUMAR, RANDHAWA, NIRUPAM, et al., 2014).

O uso generalizado de antibióticos de amplo espectro por períodos prolongados favorece a pressão seletiva sobre a microbiota hospitalar, contribuindo para o surgimento de cepas de *A. baumannii* multirresistentes, restringindo as opções terapêuticas na clínica médica (SHENG, LIAO, LAUDERDALE, et al., 2010). Dentre os mecanismos de resistência, destacam-se a reduzida permeabilidade da membrana externa, alteração nos sítios de ligação dos antibióticos, hiperexpressão de bombas de efluxo e produção de β-lactamases. Estas últimas são consideradas uma grande preocupação, devido à sua rápida capacidade de disseminação (PELEG, SEIFERT, PATERSON, 2008; MOSTACHIO, LEVIN, RIZEK, et al., 2012; MARTINS, BARTH, 2013; ABDALHAMID, HASSAN, ITBAILEH, et al., 2014).

Embora algumas enzimas metalo-β-lactamases (MβLs) já tenham sido descritas, as oxacilinases (OXAs) codificadas pelo gene $bla_{\text{OXA-like}}$, são as enzimas de maior frequência em cepas de *A. baumannii* (ABDALHAMID, HASSAN, ITBAILEH, et al., 2014), sendo OXA-

23, OXA-24, OXA-51 e OXA-58 as mais relatadas (MOSTACHIO, LEVIN, RIZEK, et al., 2012; KAMOLVIT, SIDJABAT, PATERSON, 2015). No entanto, além dos múltiplos mecanismos de resistência, cepas de *A. baumannii* apresentam uma rápida disseminação devido à sua fácil adaptação no ambiente e pela associação dos genes de resistência à plasmídeos, transposons, integrons e sequências de inserção (HOWARD, O'DONOGHUE, FEENEY, et al., 2012; TANGA, APISARNTHANARAKB, HSUC, 2014).

No estado do Mato Grosso do Sul (MS) não existem relatos sobre o monitoramento de cepas de *A. baumannii* multirresistentes, desta forma, o objetivo deste estudo foi avaliar o perfil epidemiológico e molecular de cepas de *A. baumannii* resistentes a carbapenêmicos isoladas em Unidades de Terapia Intensiva Neonatal e Adulto de um hospital público de Dourados/MS, visando identificar os fatores de risco relacionados à infecção e colonização ocasionadas por este microrganismo. O desenvolvimento deste trabalho visa auxiliar no monitoramento da ocorrência de cepas de *A. baumannii* resistentes a carbapenêmicos, contribuindo para delinear a amplitude do problema dentro do ambiente hospitalar.

2. Revisão de literatura

2.1 Infecção hospitalar

Infecções hospitalares ou Infecções Relacionadas à Assistência à Saúde (IRAS) constituem um problema público, sendo reconhecidas como uma das principais causas de morbidade e mortalidade, estabelecendo para o sistema de saúde, altos custos com internação e uso de medicamentos. Além disso, proporcionam um grande impacto clínico, uma vez que os pacientes internados estão expostos a uma ampla variedade de microrganismos patogênicos, intervindo na segurança dos pacientes e na qualidade dos serviços de saúde (SYDNOR; PERL, 2011; GARCIA, CÉSAR, BRAGA, et al., 2013).

O controle das infecções nosocomiais se torna de difícil conduta devido às condições que os próprios pacientes apresentam principalmente os internados em UTIs, os quais estão sujeitos a prolongados períodos de internação, uso de antibióticos de amplo espectro, realização de procedimentos invasivos, doenças de base, comprometimento do sistema imunológico, contaminações cruzadas e infecções endógenas. Os fatores agravantes associados às UTIs contribuem para o aumento das taxas de infecção neste local em relação às demais unidades de internação, sendo as infecções urinárias, respiratórias, de sítios cirúrgicos, sanguíneas, gastrointestinais e de pele as mais frequentes (OLIVEIRA, KOVNER, SILVA, 2010; SYDNOR; PERL, 2011).

Rosenthal e colaboradores (2013) avaliaram a prevalência das infecções de sítios cirúrgicos em 82 hospitais de 30 países que participam do *International Nosoconial Infection Control Consortium* (INICC), no período de janeiro de 2005 a dezembro de 2010, abrangendo os 4 continentes (América, Ásia, África e Europa). As maiores taxas de infecção de sítios cirúrgicos foram a derivação ventricular (12, 9%), cirurgia do colón (9, 4%) e cirurgia pancreática, fígado ou ducto biliar (9, 2%). As cirurgias da tireoide ou da paratireoide (0, 3%) e parto cesariano (0, 7%) foram consideradas as cirurgias que apresentaram as menores taxas de infecção. Os maiores índices de infecção hospitalar foram associados ao uso de dispositivos invasivos, ao tipo de hospital (público, acadêmico ou privado) e ao nível socioeconômico do país. Entretanto, em muitos hospitais houve a redução de 30 a 70% das incidências de infecções a partir da implementação de processos de vigilância e programas de controle de infecção hospitalar.

Segundo a Organização Mundial da Saúde (OMS, 2016), 234 milhões de pacientes são submetidos a cirurgias por ano, sendo que destes, 1 milhão morrem em decorrência de infecções hospitalares e 7 milhões apresentam complicações no pós-operatório. Em países desenvolvidos, 5 a 15% dos pacientes internados em enfermarias e 50% dos pacientes internados em UTIs são acometidos por algum quadro de infecção. Nos países em desenvolvimento as taxas podem variar de 75% a 88, 9% nas UTIs Neonatal e Adulto (WHO, 2016), uma vez que estes países apresentam deficiências nos sistemas de cuidados de saúde, agravados ainda mais pelos problemas econômicos, estrutura hospitalar, superlotações e falta de políticas de controle de infecção adequadas (ALLEGRANZI, NEJAD, COMBESCURE, et al., 2011).

Aproximadamente 4 milhões de pacientes são vulneráveis a adquirir uma infecção associada aos cuidados de saúde na Europa por ano, sendo que 37 mil mortes estão diretamente relacionadas a algum tipo de infecção. Outro fator importante, é que aproximadamente 20 a 30% das infecções hospitalares são consideradas evitáveis a partir da implementação de programas de higiene e controle intensivo dessas (ECDC, 2016).

Nos Estados Unidos, a cada 25 pacientes 1 apresenta algum tipo de infecção associada aos cuidados de saúde, sendo que 722 mil infecções hospitalares foram notificadas no ano de 2011 e 75 mil pacientes morreram durante a internação (CDC, 2016). No Brasil, estima-se que as taxas de infecção hospitalar atinjam de 14 a 19% das internações. A cada 100 pacientes internados, 13 adquirem algum tipo de infecção durante o período de internação e aproximadamente 100 mil pacientes morrem por ano em decorrência de quadros infecciosos. Este risco aumenta ainda mais nas UTIs, onde 30 a 47% dos pacientes adquirem infecções após a admissão nestas unidades (OMS, 2016).

Neste contexto, algumas medidas são necessárias em busca do controle de infecções dentro dos ambientes nosocomiais. Restrição no uso de antibióticos de amplo espectro, cuidados na higienização de pacientes e alas hospitalares, realização de uma vigilância clínica e epidemiológica e programas de controle de infecção hospitalar são algumas das medidas que podem ser utilizadas. A implementação destas medidas visa a prevenção de novos casos de infecção, além de contribuir para o aumento do índice de sobrevida e redução de gastos com internação e medicamentos (PELEG, SEIFERT, PATERSON, 2008; OLIVEIRA, KOVNER, SILVA, 2010; SYDNOR, PERL, 2011).

2.2 Acinetobacter baumannii

A nomenclatura do gênero *Acinetobacter* spp. é considerada complexa, uma vez que a primeira descrição deste gênero ocorreu em 1911, após o isolamento de um microrganismo encontrado no solo, nomeado de *Micrococcus calcoaceticus* por Beijerink, um microbiologista holandês (BEIJERINK, 1911 apud PELEG; SEIFERT; PATERSON, 2008). No entanto, com o decorrer das décadas, este gênero passou por várias outras classificações, até que em 1971 ocorreu o reconhecimento oficial do gênero *Acinetobacter* spp., após um estudo publicado por Baumann e colaboradores (1968), enquanto a espécie *A. baumannii* foi nomeada apenas em 1986 por Bouvet e Grimont (1986).

Acinetobacter spp. pertence à família *Moraxellaceae* (ROSSAU, VAN LANDSCHOOT, GILLIS, et al. 1991) e compreende cerca de 41 espécies diferentes (EUZEBY, 2016). *A. baumannii* é a espécie considerada clinicamente mais importante (PELEG, SEIFERT, PATERSON, 2008; MOLINA, CISNEROS, FERNANDEZ-CUENCA, et al., 2010; MARTINS, BARTH, 2013) (figura 1), porém, outras espécies relacionadas a casos de infecção já foram descritas na literatura, como *Acinetobacter calcoaceticus* (WEI, HSU, LIN, et al., 2014), *Acinetobacter lwoffii* (KAMOLVIT, SIDJABAT, PATERSON, 2015), *Acinetobacter pitti*, *Acinetobacter nosocomialis* (RODRÍGUEZ, NASTRO, DABOS, et al., 2014), *Acinetobacter johnsonii* (ZONG, ZHANG, 2013), *Acinetobacter haemolyticus* (FUNAHASHI, TANABE, MAKI, et al., 2013), *Acinetobacter junii* (ZHOU, GUAN, YANG, et al., 2012) e *Acinetobacter radioresistente* (HIGGINS, ZANDER, SEIFERT, 2012).

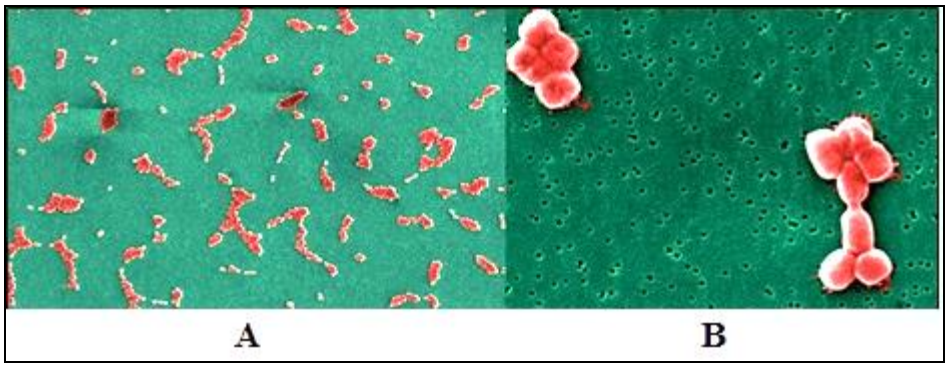

Figura 1. *Acinetobacter baumannii* observado por microscopia eletrônica de varredura. Figura A (ampliação de 1, 546x) e figura B (ampliação de 12, 739x). Adaptado de CDC (2005).

A. baumannii apresenta fatores que facilitam a colonização de pacientes em ambientes hospitalares (QI, SCHEETZ, MALCZYNSKI, 2009). Mecanismos presentes na membrana externa como lipopolissacarídeo (LPS), vesículas e proteínas, bem como, a presença da

cápsula polissacarídica, fosfolipases e sistemas de captação de ferro são considerados importantes fatores de virulência deste patógeno (GIAMARELLOU, ANTONIADOU, KANELLAKOPOULOU, 2008; MCCONNELL, ACTIS, PACHÓN, 2013) (tabela 1).

Tabela 1. Fatores de virulência envolvidos na patogenicidade de *Acinetobacter baumannii*.

Fatores de virulência (gene)	Patogenicidade	Estudos
OmpA (*OmpA*)	Indução da apoptose nas células hospedeiras, aderência e invasão das células epiteliais, formação de biofilme, motilidade superficial.	GADDY, et al., 2009
Lipopolissacarídeo (*lps*B)	Evasão da resposta imune e desencadeamento da resposta inflamatória do hospedeiro.	LUKE, et al., 2010
Cápsula polissacarídica (*ptk* e *eps*A)	Evasão da resposta imune do hospedeiro e proteção contra dessecação.	RUSSO, et al., 2010
Fosfolipase D	Disseminação bacteriana, sobrevivência bacteriana *in vivo.*	JACOBS, et al., 2010
Penicillin-binding protein (*pbp*G)	Biosíntese do peptideoglicano, estabilidade celular.	RUSSO, et al. 2009
Outer membrane vesicles (OMVs)	Entrega do fator de virulência ao citoplasma da célula do hospedeiro, transferência de material genético entre células bacterianas.	JIN, et al., 2011
Sistemas de captação de ferro (acinetobactin)	Fornecimento de ferro necessário para persistir no hospedeiro, causa apoptose celular.	GADDY, et al., 2012

Adaptado de Mcconnell e colaboradores (2013).

O estudo de Smith e colaboradores (2007), mostra que a cepa de *A. baumannii* (ATCC 17978) possui cromossomo circular, o qual contém 3.976.746 pares de base (pb), sendo composto por dois plasmídeos identificados como pAB1 (13.404 pb) e pAB2 (11.520 pb) (figura 2). Em comparação com a sequência da cepa não patogênica de *Acinetobacter baylyi* foi observado que *A. baumannii* não possuía dois importantes genes envolvidos na captação de DNA exógeno, os genes *com*P e *com*A, no entanto possuía outros genes como *com*EA, *com*EC, *pil*Q, *com*E e *pil*F, relacionados à incorporação de DNA exógeno. Estas características fazem de *A. baumannii* um importante microrganismo patogênico (LIN, LAN, 2014).

A. baumannii possui diversas ilhas de patogenicidade (*PAI-pathogenicity islands*), locais em que se encontram genes responsáveis pela resistência a antibióticos, virulência, resistência a metais pesados, absorção e metabolismo de ferro, genes fimbriais, biogênese do envelope celular, bem como, genes envolvidos no metabolismo de lipídeos, absorção de aminoácidos e processamento e degradação de xenobióticos. De modo geral, as ilhas de patogenicidade são segmentos de DNA inseridos nos cromossomos de bactérias patogênicas. Apresentam como características um ou mais genes de virulência, com tamanho de 10 a 200 kb e geralmente possuem um conteúdo de guanina mais citosina (G + C entre 39-47%)

diferente do restante do genoma bacteriano, estão normalmente associadas aos genes do RNA transportador (RNAt) e associadas a elementos genéticos móveis (SCHMIDT, HENSEL, 2004; WEI, HSU, LIN, et al., 2014).

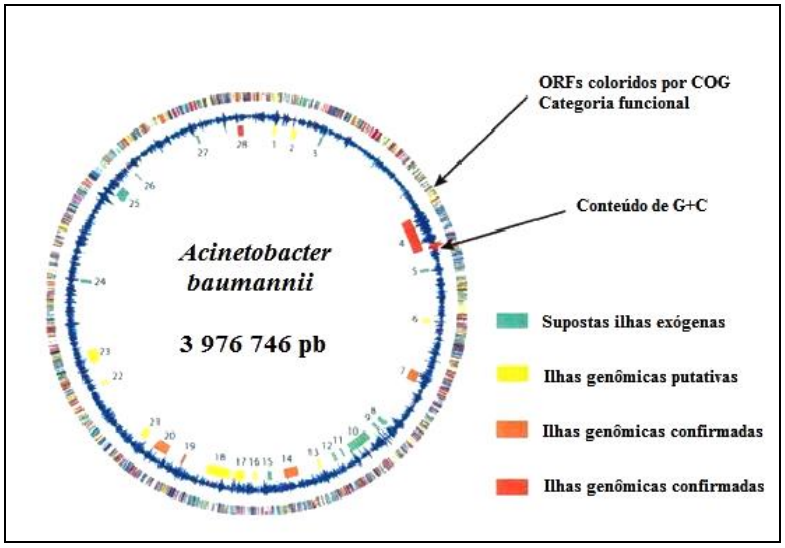

Figura 2. Mapa do genoma de *Acinetobacter baumannii*. O círculo externo mostra os genes diferenciados por cores atribuídos pelo COG (*clusters* ou *orthologous groups*). O círculo azul representa a porcentagem média de G+C. As caixas em verde indicam supostas ilhas exógenas; as amarelas indicam ilhas genômicas putativas; as vermelhas e laranjas indicam ilhas genômicas já confirmadas envolvidas na patogenicidade de *A. baumannii*. Adaptado de Smith e colaboradores (2007).

2.3 Importância clínica de infecções e colonizações ocasionadas por *Acinetobacter baumannii*

A. baumannii é considerado um importante patógeno nosocomial (HENIG, WEBER, HOSHEN, et al., 2015), envolvido em diversos casos de infecção e colonização, o que dificulta o tratamento de pacientes, considerados importantes veículos para a transmissão horizontal e disseminação de cepas multirresistentes nos ambientes hospitalares (QI, SCHEETZ, MALCZYNSKI, 2009; ARVANITI, LATHYRIS, RUIMY, et al., 2012).

As manifestações clínicas mais comuns ocasionadas por *A. baumannii* são pneumonia hospitalar associada principalmente ao uso de ventilação mecânica, infecções da corrente sanguínea, infecções urinárias, bacteremias associadas ao uso de dispositivos por longa permanência, bem como, meningites, infecções oculares, intra-abdominais, de sítios cirúrgicos, do trato respiratório e trato gastrointestinal (SHENG, LIAO, LAUDERDALE, et al., 2010; AL-ANAZI, AL-JASSER, 2014; NONAKA, NAGAE, OMAE, et al., 2014; DOI, MURRAY, PELEG, 2015). Permanece facilmente nos ambientes hospitalares, uma vez que é

considerado colonizador habitual da pele, feridas, trato respiratório e trato gastrointestinal de pacientes, além de colonizar aparelhos e dispositivos hospitalares, demonstrando a sua facilidade em interagir com diferentes tipos de superfícies (figura 3) (GIAMARELLOU, ANTONIADOU, KANELLAKOPOULOU, 2008; POUR, DUSANE, DHAKEPHALKAR, et al., 2011; EIJKELKAMP, STROEHER, HASSAN, et al., 2014).

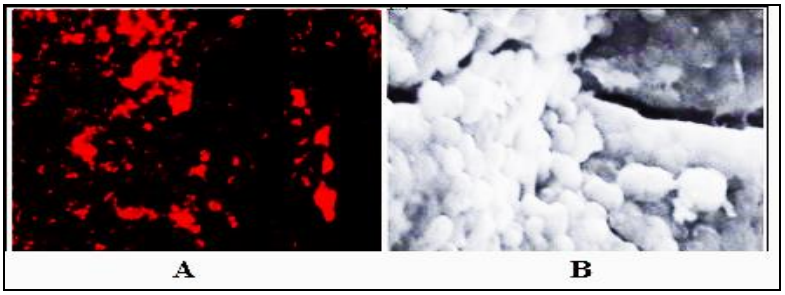

Figura 3. Formação de biofilme por *Acinetobacter baumannii*. A - células aderidas a superfície de vidro coradas com acridina laranja 0, 02% analisada por epifluorescência. B – formação de biofilme e adesão de *A. baumannii* à superfície de cateter urinário analisado por microscopia eletrônica de varredura. Adaptado de Pour e colaboradores (2011).

Avaliando o contexto clínico, a ANVISA (Agência Nacional de Vigilância Sanitária) publicou um relatório sobre as infecções primárias da corrente sanguínea relacionadas ao uso de cateter venoso central (CVC) em UTIs adulto, pediátrica e neonatal de todas as regiões brasileiras durante o ano de 2014. Aproximadamente 19.941 infecções relacionadas ao uso de CVC foram notificadas nas UTIs adulto, sendo 2.960 (12, 9%) ocasionadas por *Acinetobacter* spp. e destes, 2.346 (79, 3%) foram resistentes aos carbapenêmicos. Nas UTIs pediátricas, *Acinetobacter* spp. foi responsável por 131 (5, 6%) das 2.682 notificações de infecção, sendo que das 131 cepas isoladas, 57 (43, 5%) apresentaram resistência aos carbapenêmicos. Enquanto que nas UTIs neonatais, *Acinetobacter* spp. ocasionou 223 (3, 2%) das 11.241 infecções notificadas, e destas 223 cepas isoladas, 74 (33, 2%) foram consideradas resistentes aos carbapenêmicos. As taxas de resistência aos carbapenêmicos encontradas nas UTIs distribuídas nas 5 regiões brasileiras são mostradas na tabela 2, onde é possível verificar que a região Centro-Oeste apresentou taxas elevadas de isolamento de *Acinetobacter* spp. resistentes aos carbapenêmicos isolados nas UTIs adulto e pediátrica (BRASIL, 2015). Estes dados sugerem uma maior atenção para a realização de estudos e avaliação das medidas propostas para o controle de infecções hospitalares e resistência bacteriana em nossa região.

O estudo de vigilância SCOPE Brasileiro (*Surveillance and Control of Pathogens of Epidemiological Importance*) avaliou os dados de infecção da corrente sanguínea de 16 hospitais brasileiros no período de 2007 a 2010, totalizando 2.447 pacientes integrantes deste

estudo. A partir dos resultados obtidos foi possível observar que *Acinetobacter* spp. foi isolado em 12, 5% dos pacientes que apresentavam o quadro de infecção, bem como em 15, 2% dos pacientes internados nas UTIs e em 10% dos pacientes que apresentavam infecções da corrente sanguínea internados em outras alas hospitalares, como centro cirúrgico (13, 9%), pediatria (15, 5%), neurocirurgia (14, 1%), pediatria hematologia/oncologia (11, 7%) e cirurgia cardiotorácica (12, 8%). O tempo médio de internação para o início das infecções da corrente sanguínea por este patógeno foi de 22 dias, ao passo que a mortalidade dos pacientes internados nas UTIs foi de 65, 5%. Os níveis de resistência das cepas de *Acinetobacter* spp. isoladas foram de 55, 9% para imipenem, meropenem (56, 4%), ceftazidima (70%), cefepime (77, 7%), ciprofloxacin (73, 4%), gentamicina (51, 8%), ampicilina-sulbactam (34, 7%) e 75, 7% para piperacilina/tazobactam. Dos 112 isolados resistentes aos carbapenêmicos, 75, 9% apresentaram o gene $bla_{\text{OXA-23}}$ (MARRA, CAMARGO, PIGNATARI, et al., 2012).

O estudo SENTRY *Antimicrobial Surveillance Program* realizado entre 2008 e 2010 avaliou a prevalência de cepas de *Acinetobacter* spp. e outros bacilos Gram-negativos isolados em centros médicos latino-americanos (Argentina, Brasil, Chile e México). Neste período, 5.704 bacilos gram-negativos foram isolados, sendo 845 classificados como *Acinetobacter* spp. Este microrganismo foi responsável por 7, 2% das 6.035 infecções da corrente sanguínea, 7% dos 1.442 quadros de pneumonia e 9, 9% das 1531 infecções de pele e tecidos moles, mantendo-se entre os 5 patógenos mais isolados durante o período do estudo. O perfil de resistência foi avaliado em 845 cepas de *Acinetobacter* spp., as quais apresentaram resistência frente a todos os antibióticos testados: imipenem (67, 8%), meropenem (66, 1%), ceftriaxona (55, 6%), ceftazidima (81, 7%), cefepima (76, 6%), ciprofloxacina (87, 2%), amicacina (62, 6%), gentamicina (53, 3%), tobramicina (43, 4%), piperacilina/tazobactam (86, 3%) e colistina (1, 2%). As oxacilinases encontradas nesse estudo foram OXA-23 e OXA-24 na Argentina, OXA-23 no Brasil, OXA-58 no Chile e OXA-24 no México (GALES, CASTANHEIRA, JONES, et al., 2012).

Tabela 2. Taxa de infecções relacionadas ao uso de cateter venoso central ocasionadas por *Acinetobacter* spp. resistentes aos carbapenêmicos nas Unidades de Terapia Intensiva adulto, pediátrica e neonatal no ano de 2014 de acordo com a região geográfica brasileira.

Regiões brasileiras	**UTIs adulto**			**UTIs pediátrica**			**UTIs neonatal**		
	Infecções notificadas	***Acinetobacter* spp.**	**Resistentes aos carbapenêmicos**	**Infecções notificadas**	***Acinetobacter* spp.**	**Resistentes aos carbapenêmicos**	**Infecções notificadas**	***Acinetobacter* spp.**	**Resistentes aos carbapenêmicos**
Centro-Oeste	1.994	189 (9, 5%)	156 (82, 5%)	238	8 (3, 4%)	4 (50, 0%)	703	21 (3, 0%)	4 (19%)
Sudeste	13.670	1.971 (14, 4%)	1.611 (81, 7%)	1.462	87 (6, 0%)	42 (48, 3%)	4.127	117 (2, 8%)	42 (35, 9%)
Sul	2.649	234 (8, 8%)	182 (77, 8%)	282	12 (4, 3%)	1 (8, 3%)	716	9 (1, 3%)	1 (11, 1%)
Nordeste	3.536	479 (13, 5%)	338 (70, 6%)	295	20 (6, 8 %)	10 (50, 0%)	890	26 (2, 9%)	1 (3, 8%),
Norte	1.140	87 (7, 6%)	59 (67, 8%)	83	4 (4, 8%)	0 (0%)	466	50 (10, 7%)	26 (52%)
Total	**19.941**	**2.960 (12, 9%)**	**2.346 (79, 3%)**	**2.682**	**131 (5, 6%)**	**57 (43, 5%)**	**11.241**	**223 (3, 2%)**	**74 (33, 2%)**

Adaptado de (BRASIL, 2015).

2.4 Fatores de risco relacionados às infecções e colonizações ocasionadas por *Acinetobacter baumannii*

Fatores de risco relacionados à aquisição de *A. baumannii* multirresistentes estão envolvidos diretamente com o aumento da susceptibilidade de pacientes internados desenvolverem algum tipo de quadro infeccioso, resistência bacteriana e consequentemente, casos de mortalidade nos ambientes nosocomiais. A investigação dos fatores de risco envolvidos em casos de infecções e colonizações ocasionadas por *A. baumannii* contribui para a prevenção e controle da resistência bacteriana, desta forma, diversos estudos passaram a ser relatados, a fim de contribuir para o controle deste patógeno nos ambientes nosocomiais, principalmente nas UTIs adulto, pediátrica e neonatal (HENIG, WEBER, HOSHEN, et al., 2015) (tabela 3 e 4).

Nas UTIs adulto vários são os fatores envolvidos na aquisição de infecções e colonizações ocasionadas por *A. baumannii*. Fatores como procedimentos invasivos, uso de ventilação mecânica, cateter venoso central e cateter urinário >6 dias, dreno, tubo endotraqueal, traqueostomia, tubo de gastrostomia, tempo de permanência hospitalar entre 3 e 7 dias na UTI, hospitalizações recorrentes, transferência de outro hospital, uso de corticoides, quimioterapia, uso prévio de antibióticos, transplante de órgãos, doenças crônicas, bacteremias recentes, tumor, doenças hematológicas, falência respiratória e cardiovascular e quadro de pneumonia são indicados como de risco para a aquisição deste patógeno nas UTIs adulto. Alguns estudos associam estes fatores com o desenvolvimento de quadros de pneumonia, infecções da corrente sanguínea e quadros de bacteremias (ROCHA, VILELA, CEZÁRIO, et al., 2008; NUTMAN, GLICK, TEMKIN, et al., 2014; CHUSRI, SILPAPOJAKUL, MCNEIL, et al., 2015; HENIG, WEBER, HOSHEN, et al., 2015).

Recém-nascidos são considerados susceptíveis para o desenvolvimento de infecções e colonizações por *A. baumannii*, principalmente quadros de infecção da corrente sanguínea ou respiratória, pneumonia, bacteremias e sepse nas UTIs neonatais. Diversos estudos relatam como fatores de risco em neonatos, infecção materna, prematuridade, peso ao nascer <2500 gramas, internação anterior, permanência na UTI >3 dias, síndromes respiratórias, doenças hematológicas, neutropenia >3 dias, lesão cerebral traumática, transfusão sanguínea, procedimento cirúrgico, alimentação parental, reintubação, ventilação mecânica, uso de cateter venoso central, cateter umbilical e uso prévio de antibióticos (VON DOLINGER DE BRITO, OLIVEIRA, ABDALLAH, et al., 2005; ZARRILLI, DI POPOLO, BAGATTINI, et al., 2012; THATRIMONTRICHAI, APISARNTHANARAK, CHANVITAN, et al., 2013,

KUMAR, RANDHAWA, NIRUPAM, et al., 2014, REDDY, MORROW, MARGENT, 2015).

A prevalência de casos de infecção e colonização ocasionadas por *A. baumannii* são maiores em UTIs, como ocorre nas UTIs adulto (HENIG, WEBER, HOSHEN, et al., 2015), uma vez que neste local concentram-se pacientes clínicos ou cirúrgicos mais graves, além disso, apresentam um sistema imunológico comprometido decorrente de outras comorbidades, estado nutricional alterado, internação prolongada, além de serem submetidos a procedimentos invasivos, uso de drogas imunossupressoras e antibióticos de amplo espectro. Pacientes idosos são considerados ainda mais susceptíveis, pois apresentam deficiência imunológica (OLIVEIRA, KOVNER, SILVA, et al., 2010; MARTINS, BARTH, 2013).

Recém-nascidos são também considerados susceptíveis para o desenvolvimento de infecções e colonizações por *A. baumannii*. Diversos estudos relatam vários fatores de risco em UTIs neonatais, uma vez que neonatos apresentam o sistema imunológico em maturação, barreiras da pele e mucosas são ineficientes às intervenções terapêuticas, como o uso de dispositivos invasivos, antibióticos de amplo espectro, uso de imunossupressores e corticoides. O quadro dos recém-nascidos se agrava ainda mais quando são neonatos prematuros (<28 semanas) e de baixo peso (<2.500 gramas), que devido às suas condições apresentam uma longa permanência no hospital (DAS, SINGH, PAL, et al., 2011; ROMANELLIA, ANCHIETA, MOURÃO, et al., 2013; WEI, HSU, LIN, et al., 2014).

Tabela 3. Fatores de risco associados à infecção e colonização ocasionadas por *Acinetobacter baumannii* em Unidades de Terapia Intensiva adulto.

Estudos	Local do estudo	Período do estudo	No. de pacientes	Casos	Controles
JANG, et al., 2009	Taiwan	1997-2006	154	77 pacientes com infecção da corrente sanguínea por *AB.*	77 pacientes com infecção da corrente sanguínea sem *AB.*
YE, et al., 2010	Alemanha	2001-2005	209	49 pacientes com MDRAB.	160 pacientes com CSAB.
ROCHA, et al., 2008	Brasil	2005-2006	275	84 pacientes com PAVM.	191 sem PAVM.
BROTFAIN, et al, 2016	Israel	2005-2011	129	46 pacientes com pneumonia e cultura de escarro positiva para MDRAB 72 hs após o início de VM e quadro de bacteremia.	83 pacientes com pneumonia e cultura de escarro positiva para MDRAB 72 hs após o início de VM, sem desenvolver bacteremia.
ELLIS, et al., 2015	Estados Unidos	2006-2012	671	302 pacientes com infecções ocasionadas por MDRAB.	369 pacientes com infecções ocasionadas por CSAB.
HENIG, et al., 2015	Israel	2007-2012	2380	1190 pacientes com CRAB.	1190 pacientes sem *AB.*
JUNG, et al., 2010	Coréia do Sul	2008-2009	200	108 pacientes com bacteremias ocasionada por *AB.*	92 pacientes sem apresentar bacteremias.
NUTMAN, et al., 2014	Israel	2008-2011	172	83 pacientes com bacteremias que morreram dentro de 14 dias.	89 pacientes com bacteremias que sobreviveram mais de 14 dias.
CHUSRI, et al., 2015	Tailândia	2010-2011	394	139 pacientes com CRAB.	197 pacientes sem *AB* e 58 pacientes com CSAB.
TSAKIRIDOU, et al., 2014	Grécia	2010-2012	193	22 pacientes com pneumonia por *AB* associada a VM.	24 pacientes com pneumonia por outros microrganismos associada a VM e 147 pacientes sem pneumonia.
MOGHNIEH, et al., 2016	Líbano	2012-2013	257	40 pacientes com *AB.*	217 pacientes sem *AB.*
GUO, et al., 2016	China	2012-2015	87	64 pacientes com infecção da corrente sanguínea por MDRAB.	23 pacientes com infecção da corrente sanguínea por CSAB.

AB - *A. baumannii*; MDRAB - *A. baumannii* multirresistente; PAVM - pneumonia associada a ventilação mecânica; VM - ventilação mecânica; CRAB - *A. baumannii* resistentes a carbapenêmicos; CSAB -*A. baumannii* sensíveis a carbapenêmicos, hs – horas.

Tabela 4. Fatores de risco associados à infecção e colonização ocasionadas por *Acinetobacter baumannii* em Unidades de Terapia Intensiva pediátrica e neonatal.

Estudos	Local do estudo	Período do estudo	No. de pacientes	Casos	Controles
VON DOLINGER de BRITO, et al., 2005	Brasil	2001-2002	33	11 pacientes com quadros infecciosos ocasionados por *AB.*	22 pacientes sem quadros infecciosos ocasionados por *AB.*
DENG, et al., 2011	China	2002-2008	349	117 pacientes com PAVM por *AB.*	232 pacientes sem PAVM por *AB.*
HSU, CHU, LIEN, et al., 2014	Taiwan	2004-2010	248	37 pacientes com bacteremia por *AB.*	74 pacientes sem bacteremias e 137 pacientes com bacteremia por *E. coli* ou *Klebsiella* spp.
PUNPANICH, et al., 2012	Tailândia	2005-2010	176	91 pacientes com bacteremia por CRAB.	85 pacientes com bacteremia por CSAB.
HOSOGLU, et al., 2012	Turquia	2006-2007	192	64 pacientes com sepse por *AB.*	128 pacientes com amostras de sangue sem *AB.*
De OLIVEIRA COSTA, et al. 2015	Brasil	2009-2012	101	47 pacientes com quadros infecciosos ocasionados por BGN.	54 pacientes sem quadros infecciosos ocasionados por BGN.
THATRIMONTRICHAI, et al., 2013	Tailândia	2009-2014	101	63 pacientes com pneumonia por CRAB e 13 por CSAB.	25 pacientes com pneumonia sem crescimento bacteriano ou ocasionada por outros microrganismos.
REDDY, et al., 2015	África do Sul	2010	388	194 pacientes com cultura de sangue ou amostra respiratória positiva para *AB.*	194 pacientes com cultura de sangue ou amostra respiratória negativa para *AB.*
ZARRILLI, et al., 2012	Itália	2010-2011	161	22 pacientes com *AB.*	139 pacientes sem *AB* nas primeiras 48hs.
TRAN, et al., 2015	Vietnã	2010-2011	2555	69 pacientes com sepse ocasionada por *AB.*	2486 pacientes sem quadro de sepse.
KUMAR, et al., 2014	Índia	2010-2012	65	33 pacientes com infecção da corrente sanguínea por CRAB.	32 pacientes com infecção da corrente sanguínea por CSAB.
WEI, et al., 2014	Taiwan	2010-2013	59	12 mortes por sepse ocasionada por MDRAB.	47 mortes por sepse ocasionada por outros microrganismos.

AB - *A. baumannii*; PAVM - pneumonia associada a ventilação mecânica; CRAB - *A. baumannii* resistentes a carbapenêmicos; CSAB -*A. baumannii* sensíveis a carbapenêmicos; BGN - Bacilo Gram-Negativo; MDRAB - *A. baumannii* multirresistente; *E. coli – Escherichia coli*.

2.5 Mecanismos de resistência de *Acinetobacter baumannii* aos betalactâmicos

O aumento na prevalência de infecções ocasionadas por bactérias multirresistentes em ambientes hospitalares tem emergido rapidamente a nível mundial (SANTANA, VIANA, SANTIAGO, et al., 2014). A resistência bacteriana desenvolve-se como uma consequência natural relacionada à habilidade dos microrganismos se adaptarem à pressão seletiva exercida pelo uso generalizado de diversas classes antimicrobianas por períodos prolongados, favorecendo desta forma, a seleção de microrganismos multidroga resistentes (MDR) ou até pandroga resistentes (PDR) (NEONAKIS, SPANDIDOS, PETINAKI, 2011; PORTUGAL, 2014). Estima-se que 500 mil a 1 milhão de mortes ocorram no mundo em decorrência de infecções ocasionadas por microrganismos multirresistentes. O uso de antibióticos nos ambientes hospitalares chegou a 40% na última década, sendo que a estimativa para 2050 é de 10 milhões de mortes decorrentes de microrganismos MDR (AMIB, 2015).

Várias espécies bacterianas têm sido relatadas na clínica médica apresentando um perfil de resistência frente a diferentes tratamentos antimicrobianos (PORTUGAL, 2014). As bactérias gram-negativas normalmente envolvidas em infecções hospitalares são *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae*, *Serratia marcescens*. No entanto, cepas de *A. baumannii* têm assumido um importante papel como patógenos oportunistas e de importância clínica na multirresistência e quadros de morbidade e mortalidade (PELEG, SEIFERT, PATERSON, 2008; SCHIMITH BIER, LUIZ, SCHEFFER, et al., 2010; MARTINS, BARTH, 2013).

Os mecanismos de resistência aos antibióticos podem ser de origem intrínseca ou adquirida. A resistência intrínseca ocorre sem uma exposição prévia a um antibiótico, ou seja, é uma característica da espécie bacteriana (DAVIES, DAVIES, 2010). Em *A. baumannii* pode ocorrer a partir da expressão de genes bla_{AmpC} e $bla_{\text{OXA-51}}$. A cefalosporinase cromossomal AmpC confere baixos níveis de resistência a ampicilina, no entanto, quando há a presença das sequências de inserção IS*Aba1* ou IS*Aba125*, esta enzima passa a expressar resistência a cefalotina, piperacilina, cefotaxima e ceftazidima. Enquanto que o gene $bla_{\text{OXA-51}}$ hidrolisa fracamente as classes dos antibióticos penicilinas e carbapenêmicos, mas quando mediado por IS*Aba1*, os níveis de resistência são aumentados (TIAN, ADAMS-HADUCH, TARACILA, et al., 2011; LOPES, AMYES, 2012).

Já a resistência adquirida resulta de uma alteração fisiológica ou estrutural na bactéria e ocorre quando o microrganismo sofre pressão seletiva a determinado antibiótico, ocorrendo a seleção de cepas resistentes (PORTUGAL, 2014). Pode ocorrer por mutação num *loci* do

cromossomo ou por transferência horizontal de genes (transdução, transformação e conjugação) (TANGA, APISARNTHANARAKB, HSUC, 2014). Dentre os mecanismos de resistência aos antibióticos β-lactâmicos, destaca-se a reduzida permeabilidade da membrana externa, alteração nos sítios de ligação dos antibióticos, hiperexpressão de bombas de efluxo e inativação estrutural do antibiótico pela produção de enzimas beta-lactamases (PELEG, SEIFERT, PATERSON, 2008; MOSTACHIO, LEVIN, RIZEK, et al., 2012; ABDALHAMID, HASSAN, ITBAILEH, et al., 2014). Estes mecanismos são demonstrados na figura 4.

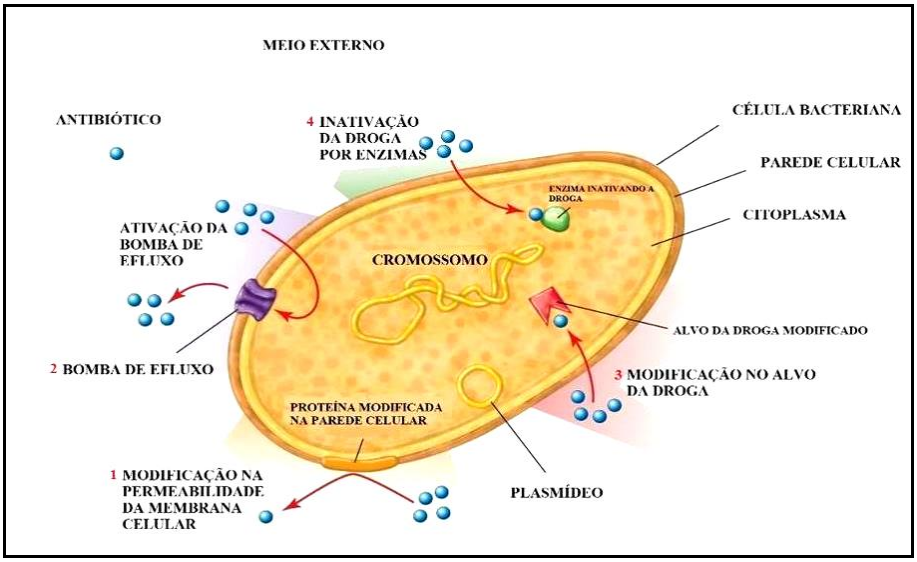

Figura 4. Desenho esquemático demonstrando os principais mecanismos de resistência bacteriana em *Acinetobacter baumannii.* 1. A perda ou diminuição da expressão dos genes codificadores de OMPs ocasiona a modificação na permeabilidade da membrana celular externa. 2. As bombas de efluxo, presentes também na membrana externa, atuam juntamente com a redução da permeabilidade da membrana, expulsando o antibiótico para fora da célula bacteriana. 3. *Penicillin binding proteins*, localizadas na membrana citoplasmática, sofrem mutações, impedindo ou modificando a ligação dos antibióticos no alvo bacteriano. 4. No espaço periplasmático, situam-se as β-lactamases, responsáveis pela hidrólise dos anéis betalactâmicos, inativando os antibióticos. Enzimas modificadoras de antibióticos, mutações ribossomais e na estrutura lipopolissacarídica são também responsáveis pela resistência bacteriana. *A. baumannii* pode abrigar integrons e transposons no cromossomo ou plasmídeos, o que auxilia na fácil disseminação da resistência bacteriana. Adaptado de Encyclopedia Britannica (2016).

Em microrganismos gram-negativos, a membrana externa é composta por fosfolipídeos, lipopolisacarídeos e *Outer Membrane Proteins* (OMPs), conhecidas como porinas, proteínas que formam canais de água no seu interior e são responsáveis pelo transporte de moléculas hidrofílicas de baixo peso molecular e pequenos metabólitos, como açúcares, aminoácidos e íons através da bicamada lipídica da membrana externa e extrusão de produtos não utilizados pela célula bacteriana. A perda ou diminuição da expressão de genes codificadores de OMPs em resposta a presença de antibióticos causa a redução da permeabilidade da membrana ou até impermeabilidade da mesma a compostos hidrofóbicos,

como antibióticos β-lactâmicos, principalmente carbapenêmicos. Desta forma, não havendo a entrada da molécula de antibiótico na célula, não ocorre morte celular bacteriana (ABBOTT, CERQUEIRA, BHUIYAN, et al., 2013; DOI, MURRAY, PELEG, 2015). Porinas denominadas CarO (29-kDa) (CATEL-FERREIRA, COADOU, MOLLE, et al., 2011), bem como, a produção diminuída de OMPs 33-36-kDa (TOMAS, BECEIRO, PEREZ, VELASCO, et al., 2005), OmpW (22-kDa) (BOU, CERVERO, DOMÍNGUEZ, 2000) e OprD (43-kDa) (DUPONT, PAGÈS, LAFITTE, et al., 2005) presentes em cepas de *A. baumannii* têm sido associadas à resistência aos carbapenêmicos. OmpA também tem sido relacionada a níveis de resistência frente ao antibiótico aztreonam, participando da extrusão deste composto a partir do espaço periplasmático associada às famílias de efluxo *Major Superfamily Facilitator* (MFS) e *Resistance Nodulation Division* (RND) (SMANI, FABREGA, ROCA, et al., 2014).

O sistema de efluxo funciona devido a atuação de OMPs e estruturas denominadas bombas de efluxo, também presentes na membrana celular bacteriana. Esse sistema é responsável pelo transporte de compostos orgânicos tóxicos do meio intracelular para o meio extracelular da célula. Portanto, quando ocorre a diminuição da permeabilidade da membrana externa e os sistemas de efluxo estão presentes, o antibiótico tem dificuldade para penetrar através da membrana externa, sendo expulso para fora do citoplasma da célula bacteriana (TORTORA, FUNKE, CASE, 2005). De modo geral, as bombas de efluxo são divididas em 6 categorias, ATP *Binding Cassette* (ABC), *Drug Metabolite Transporter* (DMT), *Major Superfamily Facilitator* (MFS), a qual contém os sistemas de efluxo TetA, TetB e CmlA e AmvA, *Multidrug and Toxic Compound Exporters* (MATE), composta pelo sistema de efluxo AbeM, *Small Multidrug Resistance* (SMR), que possui o sistema de efluxo AbeS e *Resistance Nodulation Division* (RND) formada pelo sistema de efluxo AdeABC, AdeFGH e AdeIJK (OPAZO, MELLA, DOMÍNGUEZ, 2009; COYNE, COURVALIN, PÉRICHON, 2011; LIN, LAN, 2014). Apesar da diversidade de bombas de efluxo responsáveis pela resistência aos antibióticos, como já citado anteriormente, os sistemas de efluxo AdeABC e AdeIJK (família RND) estão presentes em *A. baumannii* conferindo resistência aos β-lactâmicos, assim como o sistema de efluxo AmvA pode participar da resistência aos carbapenêmicos e cefalosporinas (DAMIER-PIOLLE, MAGNET, BRÉMONT, et al., 2008; RAJAMOHAN, SRINIVASAN, GEBREYES, 2010; COYNE, COURVALIN, PÉRICHON, 2011).

Outro mecanismo responsável pela resistência bacteriana é a alteração nos sítios de ligação dos antibióticos, este tipo de resistência ocorre por mutação nos genes cromossomais, os quais codificam enzimas com diminuição ou ausência de afinidade para o antibiótico em questão, sendo assim, o antibiótico não reconhece o alvo de ligação presente na célula

bacteriana e não se liga ao mesmo (TORTORA, FUNKE, CASE, 2005). Outro mecanismo relacionado à modificação da ligação dos antibióticos, são as enzimas *Penicillin Binding Proteins* (PBPs), localizadas na membrana plasmática, envolvidas na síntese do peptidoglicano, componente da parede celular bacteriana. Estas enzimas podem sofrer mutações, o que impedem ou modificam a ligação dos antibióticos (TORTORA, FUNKE, CASE, 2005). PBPs como PBP1a, PBP1b, PBP2, PBP3, PBP5/6, BPP6b, PBP7/8 (CAYÔ, RODRÍGUEZ, ESPINAL, et al., 2011; VASHIST, TIWARI, DAS, et al., 2011) já foram descritas em isolados de *A. baumannii*, mostrando a diversidade e complexidade dessas enzimas na resistência aos antibióticos, principalmente aos carbapenêmicos (VASHIST, TIWARI, DAS, et al., 2011).

2.5.1 Produção de betalactamases

A produção de β-lactamases é considerada a forma mais frequente de resistência bacteriana aos antibióticos β-lactâmicos. São responsáveis por hidrolisarem a ligação amida do anel β-lactâmico, inativando assim, seu efeito antibacteriano (PELEG, SEIFERT, PATERSON, 2008; BUSH, JACOBY, 2010). As β-lactamases são sintetizadas em quantidades abundantes, além disso, os genes que codificam essas enzimas são transferidos de modo relativamente fácil, uma vez que podem ser transferidos por elementos genéticos móveis como plasmídeos, integrons e transposons, já que estes elementos são responsáveis pela disseminação horizontal destas enzimas entre cepas de diferentes e mesma espécies (DOI, MURRAY, PELEG, 2015). São secretadas no espaço periplasmático, onde atuam em conjunto com a barreira da permeabilidade da parede celular externa (TORTORA, FUNKE, CASE, 2005), ocasionando níveis de resistência clinicamente significativos. No entanto, a quantidade de enzima produzida, a habilidade dessa enzima em hidrolisar os β-lactâmicos e a velocidade com que o antibiótico penetra na célula são fatores que influenciarão no grau de resistência do microrganismo produtor de β-lactamases (DALMARCO, BLATT, CÓRDOVA, 2006).

As β-lactamases podem ser classificadas conforme Ambler (1980), baseado nas sequências nucleotídicas e de aminoácidos presentes nas estruturas das enzimas, dividindo-as em grupos A, B, C e D. Enzimas pertencentes aos grupos A, C e D agem por um mecanismo baseado em resíduos de serina cataliticamente ativos para inativação da droga, enquanto as da

classe B requerem zinco para sua atividade catalítica. Podem ser classificadas ainda, conforme Bush e Jacob (2010), que se basearam na estrutura molecular, propriedades bioquímicas e sequências nucleotídicas combinadas com as características funcionais e estruturais das enzimas, as quais são divididas nos grupos 1, 2, 3 e 4.

Extended Spectrum β-lactamases (ESβLs) são pertencentes à classe A de Ambler (1980) e ao grupo 2be de Bush e Jacob (2010). São responsáveis por hidrolisarem penicilinas, cefalosporinas de amplo espectro e monobactâmicos, podendo ser inibidas por inibidores de β-lactamases, como o ácido clavulânico, sulbactam e tazobactam (SOUSA JUNIOR, FERREIRA, 2004; BUSH, JACOBY, 2010). Como os genes que codificam as ESβLs geralmente estão localizados em plasmídeos, podem apresentar resistência a outras classes de antibióticos (SOUSA JUNIOR, FERREIRA, 2004). As ESβLs diferem-se entre si por substituições na sequência de aminoácidos (1 a 7). As substituições mais importantes são as mutações que conferem amplo espectro a estas enzimas. Com a evolução dos mecanismos de resistência, houve um aumento significativo no número de variantes desta classe enzimática, chegando a 370 variantes (DALMARCO, BLATT, CÓRDOVA, 2006). Estudos relatam a resistência em cepas de *A. baumannii* decorrente destas enzimas, como TEM-1 (KRIZOVA, POIREL, NORDMANN, et al., 2013), TEM-92 (ENDIMIANI, LUZZARO, MIGLIAVACCA, et al., 2007), SHV-5 (NAAS, NAMDARI, RÉGLIER-POUPET, et al., 2007), CTX-M-2 (NAGANO, NAGANO, CORDEVANT, et al., 2004), CTX-M-15 (POTRON, MUNOZ-PRICE, NORDMANN, et al., 2011), PER-1 (JEONG, BAE, KWON, et al., 2005), PER-2 (PASTERÁN, RAPOPORT, PETRONI, et al., 2006), PER-7 (BONNIN, POTRON, POIREL, et al., 2011), VEB-1 (NAAS, COIGNARD, CARBONNE, et al., 2006).

Carbapenemases pertencentes à classe A de Ambler (1980) e ao grupo 2f de Bush e Jacob (2010) são consideradas uma das famílias enzimáticas mais versáteis dentre as β-lactamases, uma vez que é capaz de hidrolisar a maioria dos antibióticos β-lactâmicos, como carbapenêmicos, penicilinas, cefalosporinas e monobactâmicos, além de serem resistentes a alguns inibidores de β-lactamases comerciais (QUEENAN, BUSH, 2007). Enzimas como KPC-2, KPC-3 KPC-4 e KPC-10 (ROBLEDO, AQUINO, SANTÉ, et al., 2010), bem como, GES-11, GES-12, GES-14 (BOGAERTS, NAAS, EL GARCH, et al., 2010) já foram descritas em *A. baumannii*.

Metalo β-lactamases (MβLs) têm sido frequentemente associadas à resistência de cepas de *A. baumannii*. São pertencentes à classe B de Ambler (1980) e ao grupo 3a de Bush e Jacob (2010). Contribuem para a resistência frente às penicilinas, cefalosporinas e carbapenêmicos, não hidrolisam os monobactâmicos e não são inibidas por inibidores de β-

lactamases, como ácido clavulânico, sulbactam e tazobactam. Enzimas *Imipenemase* (IMP), *Verona Imipenemase* (VIM), São Paulo Metallo-β-lactamase (SPM), *German Imipenemase* (GIM), *Seul Imipenemase* (SIM) e *New Delhi Metallo- β-lactamase* (NDM) compõe este grupo (MENDES, CASTANHEIRA, CARLOS, et al., 2006). As MβLs são codificadas por cassetes gênicos localizados no cromossomo ou no plasmídeo bacteriano. No entanto, com exceção da enzima SPM-1, a qual é classificada como uma enzima cromossomal, as demais MßLs adquiridas são codificadas por genes localizados em integrons de classe 1 (MENDES, CASTANHEIRA, CARLOS, et al., 2006). Enzimas VIM-1 (IKONOMIDIS, NTOKOU, MANIATIS, et al., 2008), VIM-2, VIM-3, VIM-11 (LEE, PENG, HSU, 2008), IMP-1 (TOGNIM, GALES, PENTEADO, et al., 2006), IMP-2 (NIRANJAN, SINGH, MANCHANDA, et al., 2013), IMP-10 (CAYÔ, RODRIGUES-COSTA, MATOS, et al., 2015), SIM-1 (LEE, YUM, YONG, et al., 2005), NDM-1 (CHEN, ZHOU, JIANG et al., 2011) já foram relatadas na literatura conferindo resistência a cepas clínicas de *A. baumannii*.

A classe C de Ambler (1980), pertencente ao grupo 1 de Bush e Jacob (2010) é representada pelas cefalosporinases cromossomais (AmpC). O gene que codifica a β-lactamase do tipo AmpC pode estar localizado tanto no cromossomo quanto no plasmídeo bacteriano. Na ausência dos antibióticos β-lactâmicos, AmpC é produzida em níveis baixos, no entanto, na presença de cefoxitina e, principalmente imipenem, essas enzimas passam a ser produzidas em grandes quantidades, no entanto, carbapenêmicos e cefalosporinas de quarta geração não são hidrolisados por essa enzima, mas apresentam resistência aos inibidores de beta-lactamases, como o ácido clavulânico, tazobactam e sulbactam (CORVEC, CAROFF, ESPAZE, et al., 2003; BUSH, JACOBY, 2010). As AmpCs podem ser classificadas como enzimas plasmidiais, podendo ser induzíveis, ou seja, as enzimas são produzidas em quantidades basais na ausência de betalactâmicos e em grandes quantidades quando eles estão presentes. Estas enzimas já foram descritas em espécies bacterianas como *K. pneumoniae*, *Salmonella* spp., *P. mirabilis* e *E. coli* (PHILIPPON, ARLET, JACOBY, 2002). Em *A. baumannii*, já houve um relato de uma AmpC plasmidial identificada como DHA na China (YIN, HOU, XU, et al., 2008). Ainda dentre estas enzimas, podemos encontrar AmpCs cromossomais, presentes em *A. baumannii*, que são enzimas intrínsecas desta espécie bacteriana e não induzíveis, uma vez que o gene *amp*R está ausente neste microrganismo, sendo a enzima AmpC expressa em níveis basais, no entanto, esta enzima é capaz de hidrolisar penicilinas e cefalosporinas de amplo espectro quando o elemento de inserção IS*Aba1* ou *ISAba125* estão inseridos a montante do gene $bla_{\text{AmpC.}}$ (PHILIPPON, ARLET, JACOBY, 2002; CORVEC, CAROFF, ESPAZE, et al., 2003; BUSH, JACOBY, 2010). Foi

proposto uma nomenclatura diferenciada para as AmpCs encontradas em *A. baumannii*, identificadas como *Acinetobacter-derived cefalosporinases* (ADCs) (PHILIPPON, ARLET, JACOBY, 2002). Algumas ADCs já foram descritas em *A. baumannii*, como ADC-33 (RODRIGUEZ-MARTINEZ, NORDMANN, RONCO, et al., 2010) e ADC-56 (TIAN, ADAMS-HADUCH, BOGDANOVICH, et al., 2011).

2.5.2 Carbapenemases da classe D ou oxacilinases

As oxacilinases pertencem à classe D de Ambler (1980) e ao grupo 2 df de Bush e Jacob (2010), são codificadas pelo gene *bla*$_{\text{OXAlike}}$ e normalmente apresentam hidrólise frente aos carbapenêmicos, penicilinas e cefalosporinas (hidrolisa fracamente as de terceira e quarta geração) (BUSH, JACOBY, 2010). Oxacilinases têm sido reportadas frequentemente em cepas clínicas de *A. baumannii* associadas a surtos hospitalares (MEDEIROS, LINCOPAN, 2013). Podem ser identificadas como CHDLs (*Carbapenem-Hydrolysing Class D β-lactamase*), designadas assim, por serem as carbapenemases mais frequentes em *A. baumannii*. Estão divididas em cinco subgrupos nomeados como OXA-23, OXA-24, OXA-51, OXA-58 e OXA-143 (MOSTACHIO, LEVIN, RIZEK, et al., 2012; KAMOLVIT, SIDJABAT, PATERSON, 2015), podendo apresentar de 40 a 80% de similaridade dentre os grupos enzimáticos (QUEENAN, BUSH, 2007). Podem estar localizadas no cromossomo ou em plasmídeos bacterianos, exceto o subgrupo OXA-143, considerada uma enzima plasmidial. De modo geral, estes grupos enzimáticos podem ser encontrados em outras espécies bacterianas, como *A. junii*, *A. radioresistens*, *A. pittii*, *P. mirabilis*, *K. pneumoniae*, *P. aeruginosa*, *E. coli* (EVANS, AMYES, 2014). Apresentam baixos níveis de resistência frente aos carbapenêmicos, no entanto, a presença de sequências de inserção (IS), promovem o aumento da expressão e a disseminação das oxacilinases, com exceção das enzimas OXA-24 e OXA-143 (WANG, YAN, HOU, et al., 2014).

A primeira descrição de uma cepa produtora de *A. baumannii* foi reportada na Escócia em 1985 (LYON, 1985). O gene *bla*$_{\text{OXA-23}}$ possui 19 variantes, as quais já foram detectadas em plasmídeos e cromossomos, associados a elementos genéticos como transposons e sequências de inserção. Enzimas OXA-23 (MOSQUEDA, ESPINAL, COSGAYA, et al., 2013) e algumas das suas variantes, OXA-27 (AFZAL-SHAH, WOODFORD, LIVERMORE, 2001) e OXA-133 (MENDES, 2009) (Tabela 5) já foram descritas na literatura, no entanto, a

presença de enzimas OXA-23 é considerada mais comum em *A. baumannii* (EVANS, AMYES, 2014).

O subgrupo OXA-24 (também chamado OXA-40) compreende enzimas codificadas por genes de localização cromossomal ou plasmidial e são menos disseminadas que OXA-23 (EVANS, AMYES, 2014). Sua primeira descrição ocorreu em 2000, na Espanha. Enzimas do subgrupo OXA-24/40 (MEDEIROS, LINCOPAN, 2013), OXA-25, OXA-26 (AFZAL-SHAH, WOODFORD, LIVERMORE, 2001) e OXA-72 (LU, DOUMITH, LIVERMORE, et al., 2009) (Tabela 5) são exemplos de variantes já encontradas em cepas clínicas de *A. baumannii*.

A enzima OXA-51 possui 95 variantes e confere resistência aos carbapenêmicos somente quando associado a elementos de inserção, localizados à montante do gene, agindo como forte promotor (EVANS, AMYES, 2014; KAMOLVIT, SIDJABAT, PATERSON, 2015). O gene $bla_{\text{OXA-51}}$ já foi utilizado como um marcador genético para a identificação da espécie *A. baumannii*, pois era considerado um gene intrínseco desta espécie (ABDALHAMID, HASSAN, ITBAILEH, et al., 2014; KAMOLVIT, SIDJABAT, PATERSON, 2015), no entanto, esta enzima já foi localizada em um plasmídeo (LEE, KUO, CHIANG, et al., 2012), mudando o contexto de ser uma enzima intrínseca de *A. baumannii*. Diversos estudos já relataram a presença das variantes OXA-51 (HIGGINS, PÉREZ-LLARENA, ZANDER et al., 2013) encontradas em *A. baumannii*, como OXA-64, OXA-65, OXA-66, OXA-68, OXA-70, OXA-71 (BROWN, AMYES, 2005), OXA-69 (HÉRITIER, POIREL, FOURNIER, et al., 2005), OXA-76 (BOGAERTS, NAAS, WYBO, et al., 2006), OXA-79, OXA80 (EVANS, BROWN, HAMOUDA, et al., 2007), OXA-88, OXA-91, OXA-93, OXA-94, OXA-95 (KOH, SNG, WANG, et al., 2007), OXA-104, OXA-106, OXA-112 (EVANS, BROWN, HAMOUDA, et al., 2007).

O subgrupo OXA-58 inclui as 4 variantes OXA-58, OXA-96, OXA-97, OXA-164. Apesar de ser relatada, é considerada menos prevalente em comparação com as enzimas OXA-23 e OXA-24 (MEDEIROS, LINCOPAN, 2013; EVANS, AMYES, 2014). O primeiro relato da enzima OXA-58 produzida por *A. baumannii* ocorreu na França em 2003 (MARQUÉ, POIREL, HÉRITIER, et al., 2005). Variantes de OXA-58 (MENDES, 2009), como OXA-96 (KOH, SNG, WANG, et al., 2007) e OXA-97 (POIREL, MANSOUR, BOUALLEGUE, et al., 2008) já foram descritas.

A enzima OXA-143 (OXA-143, OXA-182, OXA-231, OXA-253, OXA-255) possui atividade contra penicilinas e carbapenêmicos. Esta enzima foi descrita por Higgins e colaboradores (2009), recuperada de uma cepa clínica de *A. baumannii* isolada em um

hospital brasileiro. Apresentam resistência aos carbapenêmicos, demonstrando altos valores de Concentração Inibitória Mínima (CIM) frente a estas drogas. Assim como ocorre com outras enzimas OXAs, o subgrupo OXA-143, confere resistência aos carbapenêmicos em maiores níveis, quando associadas a outros mecanismos de resistência (EVANS, AMYES, 2014).

Além das CHDLs, há relatos de OXAs ESβLs, como OXA-10, OXA-13, OXA-14, OXA-28 dentre outras, já encontradas em cepas de *P. aeruginosa*. Estas enzimas conferem resistência a classe da ceftazidima, no entanto, há poucos estudos epidemiológicos sobre estas enzimas, e até o momento são poucos os relatos. E ainda, a enzima OXA-48 e mais 11 variantes, que tem se tornado uma preocupação emergente, devido aos altos níveis de resistência que apresenta frente aos carbapenêmicos, como imipenem e meropenem. Estas enzimas já foram relatadas em cepas de *K. pneumoniae*, em outras enterobactérias e *A. baumannii* (EVANS, AMYES, 2014).

2.6 Mecanismos envolvidos na resistência de *Acinetobacter baumannii* aos demais antibióticos

A resistência aos aminoglicosídeos por *A. baumannii* é mediada principalmente por enzimas modificadoras de aminoglicosídeos (AMEs), como acetiltransferases (AAC), adeniltransferases (ANT) e fosfotransferases (APH), sendo que as enzimas AAC são responsáveis por modificarem o grupo amino, enquanto as enzimas ANT e APH atuam no grupo hidroxila, quebrando as ligações e inativando a molécula do antibiótico (LIN, LAN, 2014). Estudos já relataram a presença destas enzimas sendo responsáveis pela resistência aos aminoglicosídeos ocasionada por *A. baumannii*, como AAC (*aacC1*, *aacC2*) (NEMEC, DOLZANI, BRISSE, et al., 2004), AAC (*aacA4*) (CHO, MOON, JIN, et al., 2009), ANT (*aadB*) (NEMEC, DOLZANI, BRISSE, et al., 2004), ANT (*aadA1*) (CHO, MOON, JIN, et al., 2009), APH (*aphA1*) (GALLEGO, TOWNER, 2001). Outro mecanismo de resistência frente a estas drogas é a produção de metilases (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*) que atuam de modo a diminuir a afinidade dos aminoglicosídeos às subunidades ribossômicas 30S (BAKOUR, ALSHARAPY, TOUATI, 2014). Os genes que codificam estas enzimas podem ser transferidos entre espécies bacterianas diferentes através de plasmídeos, transposons ou integrons de classe 1 (LIN, LAN, 2014). A expressão de bombas de efluxo também está associada à resistência aos aminoglicosídeos. Sistemas de efluxo como AdeABC (MAGNET,

COURVALIN, LAMBERT, 2001), AdeIJK (DAMIER-PIOLLE, MAGNET, BRÉMONT, et al., 2008), AmvA (RAJAMOHAN, SRINIVASAN, GEBREYES, 2010) e AbeM (COYNE, COURVALIN, PÉRICHON, 2011) já foram descritos em *A. baumannii*.

A resistência frente ao antibiótico tigeciclina por *A. baumannii* se dá pela expressão das bombas de efluxo AdeFGH, AdeABC, AdeIJK, que quando em superexpressão podem conferir sensibilidade diminuída a esta droga (COYNE, COURVALIN, PÉRICHON, 2011). No caso da classe das tetraciclinas, *A. baumannii* apresenta a expressão das bombas de efluxo identificadas como TetA e TetB, que além das tetraciclinas são responsáveis por ocasionarem resistência às minociclinas (RIBERA, RUIZ, VILA, 2003), bem como, os sistemas de efluxo AdeABC (MAGNET, COURVALIN, LAMBERT, 2001), AdeIJK (DAMIER-PIOLLE, MAGNET, BRÉMONT, et al., 2008), CmlA e AdeFGH, que quando superexpressos contribuem para a sensibilidade diminuída deste antibiótico (COYNE, ROSENFELD, LAMBERT, et al., 2010). O gene *tetM* também tem sido relatado como causa de resistência em cepas de *A. baumannii* (RIBERA, RUIZ, VILA, 2003).

A resistência à classe das polimixinas parece estar associada às modificações no lipídeo A, um componente essencial dos lipopolissacarídeos, presentes na membrana externa da célula bacteriana. Os genes *pmrA*, *pmrB*, *lpxA*, *lpxC* e *lpxD* (MOFFATT, HARPER, HARRISON, et al., 2010) estão relacionados com a modificação do lipídio A, fazendo com que ocorra deficiência na produção do LPS, desta forma o antibiótico perde a capacidade de se ligar nos alvos bacterianos e não ocorre morte celular (VANEGAS-MÚNERA, RONCANCIO-VILLAMIL, QUICENO, et al., 2014).

A resistência ocasionada por cepas de *A. baumannii* às fluoroquinolonas pode ser mediada por bombas de efluxo, como AdeABC (MAGNET, COURVALIN, LAMBERT, 2001), AdeFGH (COYNE, ROSENFELD, LAMBERT, et al., 2010), AdeIJK (DAMIER-PIOLLE, MAGNET, BRÉMONT, et al., 2008), AbeM (COYNE, COURVALIN, PÉRICHON, 2011) e AmvA (RAJAMOHAN, SRINIVASAN, GEBREYES, 2010), enquanto que o sistema de efluxo AbeS apresenta baixos níveis de resistência frente a esta droga, no entanto a presença dos genes *gyr*A e *par*C contribuem para a resistência a esta classe de antibióticos (SRINIVASAN, RAJAMOHAN, GEBREYES, 2009).

A. baumannii apresenta resistência ao antibiótico cloranfenicol, decorrente da expressão dos sistemas de efluxo AdeABC, AdeFGH, AdeIJK (MAGNET, COURVALIN, LAMBERT, 2001; COYNE, COURVALIN, PÉRICHON, 2011), CmlA e Craa (C*hloramphenicol Resistance Acinetobacter*), considerada uma bomba de efluxo intrínseca de *A. baumannii* (VANEGAS-MÚNERA, RONCANCIO-VILLAMIL, QUICENO, et al., 2014).

Os sistemas de efluxo AbeM e AbeS conferem baixos níveis de resistência ao cloranfenicol (COYNE, COURVALIN, PÉRICHON, 2011), no entanto, a presença da membrana externa OmpA pode participar na extrusão desta droga através da membrana externa em conjunto com os sistemas de efluxo das famílias MFS ou RND (SMANI, FABREGA, ROCA, et al., 2014).

Tabela 5. Resumo dos mecanismos de resistência bacteriana em *A. baumannii*.

Antibióticos	**Mecanismos de resistência**	**Proteínas/enzimas**
β-lactâmicos	β-lactamases	
	Classe A-ESBLs	CTX-M, SHV, TEM, PER, VEB
	Classe A-Carbapenemases	GES, KPC
	Classe B-Metalo β-lactamases	VIM, IMP, SIM, NDM
	Classe C-Cefalosporinase	AmpC
	Classe D-Oxacilinases	OXA-23 e suas variantes
		OXA-24/40 e suas variantes
		OXA 51 e suas variantes
		OXA-58 e suas variantes
		OXA-143 e suas variantes
	OMPs	CarO (29-kDa), OprD (22-kDa), OmpA
		33-36-kDa, OprD (43-kDa)
	Bombas de efluxo	RND – AdeABC,
	Alteração no sítio de ligação	PBPs
Aminoglicosídeos	AMEs	AAC – AAC (*aacC1*, *aacC2*, *aacA4*)
		ANT - ANT (*aadB*, *aadA1*)
		APH - APH (*aphA1*)
	Alteração no sítio de ligação	16S rRNA metilase - *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*
	Bombas de efluxo	RND - AdeABC, AdeIJK
		MFS – AmvA
		MATE – AbeM
Tetraciclinas, Glicilciclinas	Bombas de efluxo específicas	MFS - TetA, TetB
		RND - AdeABC, AdeIJK, AdeFGH
		MFS – CmlA
	Alteração no sítio de ligação	*tetM*
Fluoroquinolonas, Quinolonas	Alteração no sítio de ligação	*gyrA*, *parC*
	Bombas de efluxo	RND – AdeABC, AdeFGH, AdeIJK
		MATE - AbeM
		MFS – AmvA
Cloranfenicol	Bombas de efluxo	RND - AdeABC, AdeIJK, AdeFGH
		MFS - CmlA
		CraA
Polimixinas	Sistema regulatório duplo	*pmrA*, *pmrB*
	Lipopolissacarídeo	*lpx/a/c/d*

ESβL: *Extended-Spectrum β-lactamase*; TEM: *Temoniera*; SHV: *Sulfhydryl Variable*; CTX-M: *Cefotaxime hydrolyzing capabilities*; GES: *Guiana Extended-Spectrum*; PER: *Pseudomonas Extended Resistant*; VEB: *Vietnam Extended-Spectrum β-lactamase*; KPC: *Klebsiella pneumoniae carbapenemase*; VIM: *Verona Imipenemase*; IMP: *Imipenemase*; SIM: *Seoul Imipenemase*; NDM: *New Deli Metallo-β-lactamase*; AmpC: *Ampicillin class C β-lactamase*; OXA*: Oxacillinase*; RND: *Resistance-Nodulation-Division;* Ade: *A. baumannii multidrug-resistant efflux pump*; TetA: *Tetracycline resistant Acinetobacter*; CmlA: *Chloramphenicol resistance Acinetobacter*; MdfA: *Multidrug facilitator*; CraA: *Chloramphenicol resistance Acinetobacter*; Amva: *A. baumannii methyl viologen* and *antimicrobial resistance protein*; AbeM: *A. baumannii efflux pump* of MATE family; AbeS: *A. baumannii efflux pump* of SMR family; AME: *Aminoglycoside-Modifying Enzyme*; AAC: *Aminoglycoside acetyltransferases*; ANT: *Aminoglycoside adenyltransferases*; APH: *Aminoglycoside phosphotransferases*; *ArmA: Armillaria mellea*; CarO: *Carbapenem-associated Outer Membrane Protein*; OMP: *Outer Membrane Protein*; PBP: *Penicillin Binding Protein*; *gyr*A/*par*C: DNA g*yrase/partitioning of the nucleoid partition*. Adaptado de Lin e colaborador (2014).

2.7 Elementos genéticos móveis

Além dos múltiplos mecanismos de resistência, cepas de *A. baumannii* apresentam uma rápida disseminação dentro dos ambientes hospitalares devido à fácil aquisição de genes de resistência ocasionada por elementos genéticos móveis, como plasmídeos, sequências de inserção, transposons e integrons, os quais são propagados através da transferência horizontal de genes (TANGA, APISARNTHANARAKB, HSUC, et al., 2014).

Blackwell e colaboradores (2016) demostraram em seu estudo a presença do plasmídeo identificado como R1215, que carrega genes que conferem resistência a gentamicina, estreptomicina, canamicina, neomicina, ampicilina, cloranfenicol, sulfametoxazol e tetraciclina em um clone de *A. baumannii*. Ainda neste contexto, várias classes de integrons têm sido descritas, sendo a classe 1 considerada a mais comum entre as bactérias gram-negativas (MIRNEJAD, MOSTOFI, MASJEDIAN, 2013). As classes de integrons 1 e 2 já foram descritas na literatura, sendo encontradas em cepas clínicas de *A. baumannii* (PLOY, DENIS, COURVALIN, et al., 2000; MIRNEJAD, MOSTOFI, MASJEDIAN, 2013, NAJAR PEERAYEH, KARMOSTAJI, 2015; AZIZI, SHAKIBAIE, BADMASTI, et al., 2016).

Nigro (2015) relata em seu estudo a presença dos transposons Tn2006 e Tn2008 em cepas de *A. baumannii* produtores de $bla_{\text{OXA-23}}$ (NIGRO, 2015), enquanto Wang e colaboradores (2014) relatam além dos transposons já citados no estudo anterior, o transposons Tn2007 em cepas clínicas de *A. baumannii* também produtores de $bla_{\text{OXA-23.}}$

O aumento no nível de resistência pelas enzimas OXA carbapenemases em cepas de *A. baumannii* pode ocorrer pela presença das sequências de inserção, as quais funcionam como fortes promotores que desempenham um importante papel na expressão dos genes de resistência aos antibióticos, principalmente carbapenêmicos (ZHONG, XU, WU, et al., 2012; KAMOLVIT, SIDJABAT, PATERSON, 2015). Diferentes sequências de inserção têm sido identificadas em cepas de *A. baumannii* (IS*Aba* - *Insertion Sequence Acinetobacter baumannii*), como no estudo de Khorsi e colaboradores (2015) que mostrou a associação do gene $bla_{\text{OXA-23}}$ com as sequências IS*Aba1* e IS*Aba4* (KHORSI, MESSAI, HAMIDI, et al., 2015; CHATTERJEE, DATTA, ROY, et al., 2016). O gene $bla_{\text{OXA-51}}$ também está associado a elementos de inserção, que contribuem para o aumento da resistência aos carbapenêmicos, como as sequências IS*Aba1* e IS*Aba9* (FIGUEIREDO, POIREL, 2009). As sequências de inserção IS*Aba1*, IS*Aba2*, IS*Aba3* e IS*Aba18* também são relatadas à montante do gene $bla_{\text{OXA-58}}$ (POIREL, NORDMANN, 2006; CHATTERJEE, DATTA, ROY, et al., 2016; SUN,

LIU, CHEN, et al., 2016). Outros estudos também relatam a presença de sequências de inserção à montante do gene *bla*$_{\text{AmpC}}$, uma vez que, as enzimas codificadas por este gene são expressas em baixos níveis não afetando o perfil de suscetibilidade de antibióticos como ampicilina, cefalotina, piperacilina, cefotaxima, ceftazidima e cefepima, no entanto, as sequências de inserção IS*Aba1* e IS*Aba125* conferem o aumento da resistência a estas drogas (TIAN, ADAMS-HADUCH, TARACILA, et al., 2011; LOPES, AMYES, 2012) (figura 5).

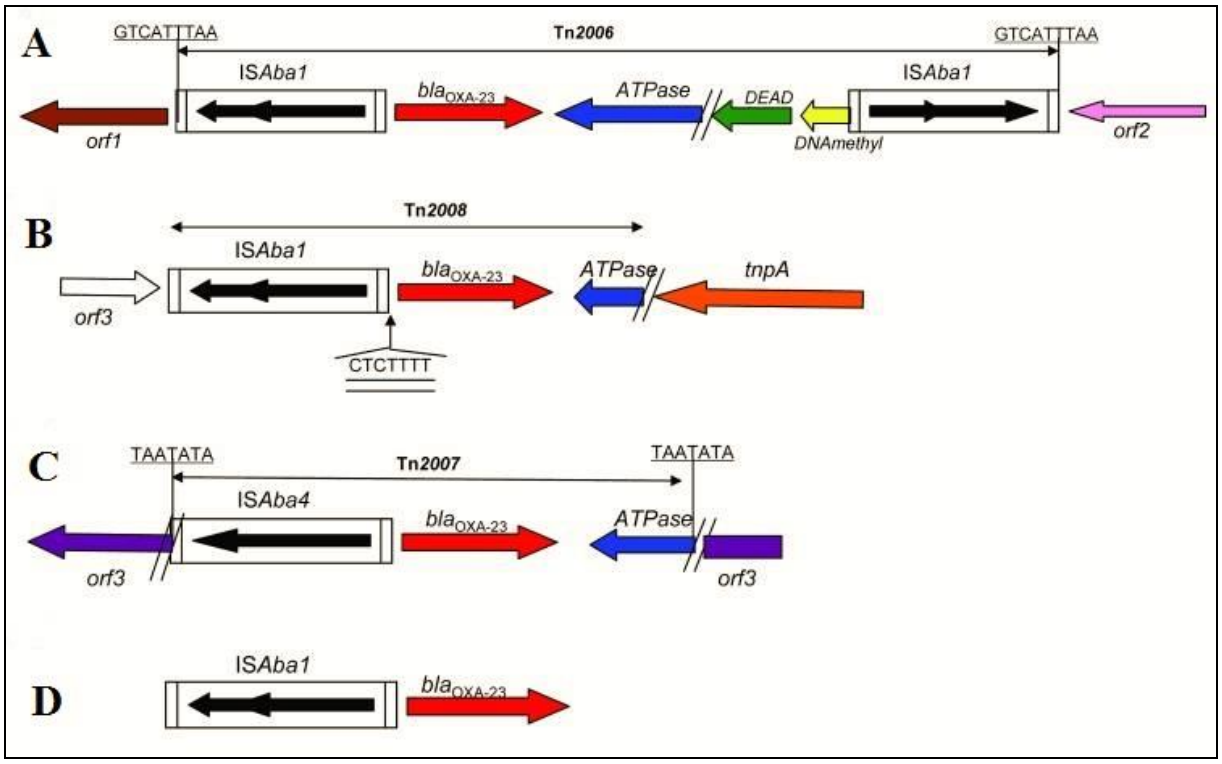

Figura 5. Representação das diferentes estruturas genéticas que abrigam o gene *bla*$_{\text{OXA-23}}$ em *Acinetobacter baumannii*. **A.** Tn2006 apresentando duas cópias do elemento IS*Aba1 upstream bla*$_{\text{OXA-23}}$. **B.** Tn2008 contendo uma cópia do elemento IS*Aba1* flanqueando o gene *bla*$_{\text{OXA-23}}$. **C.** Tn2007 com apenas uma cópia do elemento IS*Aba4 upstream bla*$_{\text{OXA-23}}$. **D.** Sequência de inserção IS*Aba*1 *upstream bla*$_{\text{OXA-23}}$. Os limites dos diferentes transposons são indicados pelos sítios de duplicação gerados provavelmente pelo mecanismo de transposição: Tn2006 (GTCATTTAA) e Tn2007 (TAATATA). O*pen Reading Frame* (ORFs 1, 2 e 3) e genes de função desconhecida são indicados. Os genes da *ATPase* e *tnpA* são responsáveis por codificarem transposases putativas. O local de inserção da sequência IS*Aba1* é indicado pelo sublinhado duplo (CTCTTTT). DEAD, gene que codifica a helicase putativa DEAD (Asp-Glu-Ala-Asp). *DNAmethyl*, DNA metilase. Adaptado de Mugnier e colaboradores (2010).

2.8 Epidemiologia molecular de *Acinetobacter baumannii*

A tipagem molecular de *A. baumannii* associada aos estudos epidemiológicos são considerados de suma importância, uma vez que proporcionam o entendimento da epidemiologia dos surtos, a identificação das transmissões cruzadas, além de auxiliar no monitoramento e no controle de infecções hospitalares (KITCHEL, RASHEED, PATEL, et al., 2009). Desta forma, diversos métodos têm sido utilizados na epidemiologia molecular de *A. baumannii* e na análise dos mecanismos envolvidos na resistência deste microrganismo frente aos antibióticos (KAMOLVIT, SIDJABAT, PATERSON, 2015).

Na Dinamarca em 2013, 8 cepas clínicas de *A. baumannii* foram isoladas. A análise genética por PFGE mostrou a presença de 4 clusters, enquanto o MLST (Plataforma Pasteur) identificou os *Sequence Type* (ST1), complexo clonal (CC1), ST2 (CC2) e ST158 (HAMMERUM, HANSEN, SKOV, et al., 2014), semelhante aos dados obtidos na França, entre 2010 e 2011, em que 110 cepas clínicas de *A. baumannii* foram isoladas. A partir da técnica de PFGE foi possível verificar a presença de 30 clusters. A análise por MLST demonstrou que as cepas apresentavam 11 STs, relatados como ST115, ST2 (CC2), ST1, ST20 e ST125 pertencentes ao CC1, ST25, ST85, ST107, ST79, ST108 e ST10 (JEANNOT, DIANCOURT, VAUX, et al., 2014).

Um estudo realizado na China durante o período de 2007 a 2009, avaliou 57 cepas de *A. baumannii* resistentes aos carbapenêmicos e positivas para os genes $bla_{\text{OXA-23}}$/IS*Aba1* e $bla_{\text{OXA-51}}$. Os 57 isolados foram classificados nos grupos clonais A e B. A técnica de MLST (Plataforma Pasteur) identificou os ST75 e ST137 (DAI, HUANG, SUN, et al., 2013). Enquanto entre 2010 e 2011, 59 cepas de *A. baumannii* produtores de $bla_{\text{OXA-23}}$ foram isoladas em um outro hospital chinês. Quinze das 59 cepas foram distribuídas em 5 perfis clonais (A-E) por PFGE. A análise das sequências dos diferentes clones permitiu a identificação dos transposons Tn2006, Tn2007 e Tn2008 (WANG, YAN, HOU, et al., 2014).

Na Árabia Saudita, entre 2012 e 2014, foram isoladas 107 cepas clínicas de *A. baumannii*. A análise por MLST (Plataforma Pasteur) resultou na presença dos ST195, ST557, ST 208, ST499, ST218, ST231, ST222 e ST286. Todos, exceto o ST231 pertencem ao CC2 (ALYAMANI, KHIYAMI, BOOQ, et al., 2015). Já nos Estados Unidos nos anos de 2008 e 2009, 65 cepas de *A. baumannii* produtores de $bla_{\text{OXA-51}}$/IS*Aba1* foram isolados de diferentes hospitais. O perfil clonal traçado pela técnica de PFGE indicou a presença de 24 clusters, enquanto que o MLST (Plataforma Pasteur) identificou os ST1, ST2, ST77, ST79, ST123, ST124, e os CC1 e CC2 (ADAMS-HADUCH, ONUOHA, BOGDANOVICH, et al.,

2011). Entre 2007 e 2012, 149 cepas de *A. baumannii* foram isoladas em diferentes hospitais do Egito, as quais apresentaram 54 clusters obtidos pela técnica de PFGE, sendo que 5 clones foram predominantes (A-E). A análise por MLST (Plataforma Pasteur) apresentou os ST763, ST777, ST369, ST762 e ST229 (BOCANEGRA-IBARIAS, PEÑA-LOPEZ, CAMACHO-ORTIZ, et al., 2015).

Um total de 94 cepas de *A. baumannii* foram isoladas de diferentes hospitais na África do Sul, durante o ano de 2008. A análise por PFGE agrupou os isolados em 4 clusters e a análise por MLST ((Plataforma Pasteur) em cincos STs (ST106, ST258, ST339, ST502, ST758, ST848), em que destes, ST258 e ST758 correspondem ao clone internacional I, ST502 e ST848 ao clone internacional II (LOWINGS, EHLERS, DREYER, et al., 2015). Na Índia, 100 cepas clínicas de *A. baumannii* foram isoladas em 2013. Foi observado um alto grau de variabilidade genética (53 padrões), sendo que apenas 18 cepas apresentaram 100% de similaridade a partir da técnica de RAPD. Os resultados obtidos por MLST (Plataforma Pasteur) identificaram as ST110, ST103, ST108, ST194, ST14, ST146, ST69, ST188, ST386, ST387, ST388, ST389, ST390 e ST391 (RYNGA, SHARIFF, DEB, 2015).

Um estudo realizado no Rio de Janeiro entre 2006 e 2007 identificou 96 cepas de *A. baumannii* produtores de $bla_{\text{OXA-23}}$. O perfil clonal destas cepas foi avaliado por PFGE e foi observado 4 perfis diferentes (A-D). Com base nos padrões de PFGE, foi observado que dois dos genótipos estavam presentes nos 8 hospitais da cidade, o que sugere a disseminação dos isolados entre estes ambientes, provavelmente devido a transferência de pacientes entre os hospitais ou através dos profissionais de saúde que atuam em mais de um hospital (CARVALHO, CARVALHO-ASSEF, PEIRANO, et al., 2009). Outro estudo também no Rio de Janeiro avaliou 177 cepas de *A. baumannii* isoladas entre 2007 e 2008. Todas as cepas apresentavam o gene $bla_{\text{OXA-51}}$ e $bla_{\text{OXA-23}}$. Foi realizado RAPD, o que identificou 28 perfis clonais, sendo os perfis B e E correspondentes ao complexo clonal (113/79) e perfil A (CC104/15). Já os resultados obtidos pelo PFGE, apresentou 5 grupos clonais (A-E). A partir do MLST (Plataforma Pasteur), foram identificados os ST79, ST156, ST15, ST25 e ST160 (MARTINS, MARTINS, DE FREITAS, et al., 2013), sendo o ST156 descrito anteriormente no Rio de Janeiro, ST25 já encontrado na Grécia, Turquia, Itália, Singapura, Árabia Saudita, Estados Unidos, Colômbia e Iraque e o ST160 já descrito no Brasil (MLST, Pasteur, 2016).

No Sul do Brasil, 585 cepas de *A. baumannii* multirresistentes foram isoladas de 12 hospitais entre 2007 a 2008. A análise do perfil clonal por REP-PCR e PFGE, mostrou a presença de 8 tipos clonais, sendo os clones identificados como 3, 4 e 11 os mais frequentes,

presentes em 5 hospitais (MARTINS KUCHENBECKER, PILGER, et al., 2012). No Sudeste brasileiro, foi relatado o isolamento de uma cepa de *A. baumannii* em 2009, a qual apresentou os genes *bla*$_{\text{OXA-23}}$ e *bla*$_{\text{OXA-51}}$. A análise por MLST (Plataforma Pasteur) indicou que a cepa pertencia ao ST15/CC15 (CHAGAS, SILVEIRA, ALBANO, et al., 2015).

Este estudo confere com um anterior, que divulgou os dois principais complexos clonais indicados como CC15 e CC79, encontrados em cepas de *A. baumannii* multirresistentes abrigando o gene *bla*$_{\text{OXA-23}}$. O CC15 já foi descrito em nove estados brasileiros, sendo Alagoas, Distrito Federal, Espírito Santo, Goiás, Minas Gerais, Rio de Janeiro, Rio Grande do Norte, Santa Catarina e Mato Grosso do Sul (CHAGAS, CARVALHO, DE OLIVEIRA SANTOS, et al., 2014). Além disso ST15 já foi descrito em outros países como Argentina e Turquia, bem como, o ST79 descrito nos Estados Unidos, Canadá e Espanha (MLST, Pasteur, 2016).

3. Considerações finais da revisão de literatura

O crescente aumento das taxas de infecções hospitalares associadas a resistência antimicrobiana por *A. baumannii* tornou-se um grande desafio à saúde pública. *A. baumannii* apresenta diversos mecanismos de resistência frente aos antibióticos, no entanto, a hidrólise por enzimas OXAs carbapenemases e metalo β-lactamases é considerada o mecanismo mais prevalente nesta espécie e representa um dos mais importantes problemas de resistência bacteriana encontrada nas cepas de interesse clínico, uma vez que conferem resistência à maioria dos antibióticos beta-lactâmicos, diminuindo, desta forma, as opções terapêuticas. Outro fator que contribui para o aumento e disseminação da resistência por *A. baumannii* é a presença de elementos genéticos móveis, como as sequências de inserção IS*Aba1* à montante dos genes oxacilinases. O monitoramento da ocorrência de cepas de *A. baumannii* de interesse clínico contribui para delinear a amplitude do problema, bem como para determinar os mecanismos de resistência bacteriana dentro das UTIs neonatal, pediátrica e adulto.

4 Objetivos

4.1 Objetivo geral

Avaliar o perfil epidemiológico e molecular de cepas de *A. baumannii* resistentes a carbapenêmicos isoladas nas Unidades de Terapia Intensiva Neonatal e Adulto de um hospital público de Dourados/MS, visando identificar os fatores de risco relacionados à infecção e colonização ocasionadas por este microrganismo.

4.2 Objetivos específicos

- Identificar cepas de *A. baumannii* de amostras clínicas de pacientes internados em Unidades de Terapia Intensiva Neonatal e Adulto em um hospital público de Dourados/Mato Grosso do Sul;
- Avaliar o perfil de resistência antimicrobiana e determinar a Concentração Inibitória Mínima (MIC);
- Avaliar a produção de carbapenemases;
- Avaliar a relação genética e ancestralidade das cepas de *A. baumannii* através do PFGE e MLST, respectivamente;
- Avaliar os fatores de risco associados às colonizações ocasionadas por *A. baumannii* na UTI Neonatal;
- Avaliar os fatores de risco associados às infecções e colonizações ocasionadas por *A. baumannii* na UTI Adulto.

5. Metodologia Geral

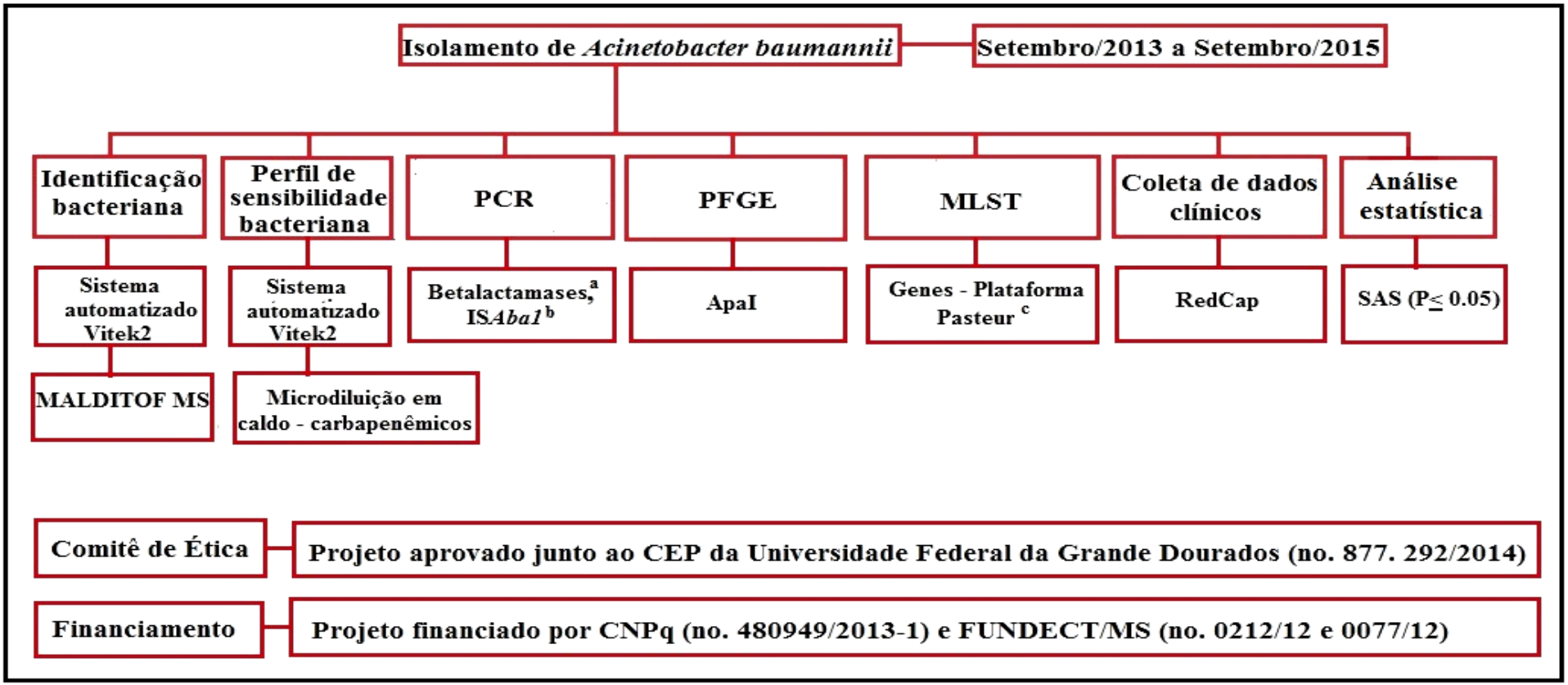

Figura 6. Metodologia geral do estudo. PCR – *Polymerase Chain Reaction*; PFGE – *Pulsed Field Gel Electrophoresis*; MLST – *Multilocus Sequence Typing*; MALDI-TOF MS - *Matrix Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry*, Apa - *Acetobacter pasteurianus* (enzima de restrição); RedCap - *Research Electronic Data Capture*; SAS - *Statistical Analysis System*; CEP – Comitê de Ética em Pesquisa; CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico; FUNDECT/MS - Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado do Mato Grosso do Sul. a. Genes das betalactamases: bla_{KPC-2}, bla_{IMP-1}, bla_{VIM-1}, bla_{NDM-1}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48}, bla_{OXA-51}, bla_{OXA-58}; b. Sequência de inserção IS*Aba1*; c. Genes MLST *Acinetobacter baumannii*: *cpn*60, *fus*A, *glt*A, *pyr*G, *rec*A, *rpl*B, *rpo*B.

6. Referências

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7 Manuscrito a ser submetido na Revista *Journal of Hospital Infection*

Outbreak caused by carbapenem-resistant *Acinetobacter baumannii* isolated from newborns in ICU in Brazilian hospital

Running title: Carbapenem-resistant *A. baumannii* in newborns

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ABSTRACT

Acinetobacter baumannii has been associated with high rates of infection and colonization in Neonatal Intensive Care Units (NICU). We describe an outbreak caused by carbapenem-resistant *A. baumannii* (CRAB) isolated from newborns. Twenty-one CRAB were isolated from newborns and presented IS*Aba1* upstream $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes. Nineteen CRAB were clonally related and sequence type 1 (ST1) was predominant. All newborns were premature and were administered beta-lactam and peripheral access. The prevalence of CRAB in NICU shows the importance of infection control measure implementation and use of stricter antibiotic policies.

Keywords: Colonization, multi-drug resistant, carbapenem, NICU.

INTRODUCTION

Acinetobacter baumannii is an opportunistic bacterial pathogen responsible for serious hospital infections and contributes to higher mortality and morbidity rates. The global emergence of carbapenem-resistant *A. baumannii* (CRAB) increases the prevalence of nosocomial infections and is becoming a major concern among neonatal and paediatrics units. Premature birth and low weight contribute to colonization and infection by *A. baumannii*. The newborns innate defence mechanisms are inefficient against therapeutic interventions such as the use of invasive devices and antimicrobial broad-spectrum. [1]

A. baumannii have several resistance mechanisms, including the low permeability of the outer membrane, alteration in antibiotic binding sites, overexpression of efflux pumps and production of carbapenemase enzymes. Metallo β-lactamase (IMP, VIM) and oxacillinases (OXA-23, OXA-24, OXA-51 and OXA-58) are more frequent in *A. baumannii*, since the carbapenemase encoding genes generally reside on transposons and integrons carried by

conjugative plasmids, increasing potential to spread. [2] This study describes the molecular characteristics and risk associated with colonization by CRAB isolated from newborns and the control measures implemented to contain the outbreak.

MATERIAL AND METHODS

Case-control study

Case-control study was conducted in the Neonatal Intensive Care Unit (NICU) and Neonatal Intermediate Unit (NIU). Newborns hospitalized between September 2013 and September 2015 at a public hospital located in the city of Dourados, Mato Grosso do Sul (a Central-Western Brazilian state) were included in this study. A case was defined as a newborn colonized by carbapenem-resistant *A. baumannii* and control were newborn presenting carbapenem-sensitive *A. baumannii*. For each case, one control was selected in the same study period, matched by age, clinical manifestation and hospital ward.

Bacterial isolates

The CRAB were obtained from 3 newborns collected from catheter tip and 18 rectal swabs. Colonization was defined as the isolation of strains without clinical manifestation of infection. [3] The study was conducted with the approval of the Research Ethics Committee from the Universidade Federal da Grande Dourados (no. 877.292/2014).

Bacterial identification, susceptibility testing and phenotypic assays

Bacterial species were identified using the Vitek®2 (bioMérieux, Hazelwood, MO) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). [4] Antimicrobial susceptibility profile was determined by Vitek®2 and interpreted according to the Clinical and Laboratory Standards Institute (CLSI/2016) guidelines. For

tigecycline, European Committee on Antimicrobial Susceptibility Testing (EUCAST/2016) was used.

Polymerase Chain Reaction amplification

Screening for the presence of carbapenemases was performed by ertapenem hydrolysis using MALDI-TOF MS.[4] Presence of β-lactamase genes (*bla*$_{\text{IMP-1}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{VIM-1}}$, *bla*$_{\text{KPC-2}}$, *bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{OXA-51}}$, *bla*$_{\text{OXA-58}}$) and IS*Aba1* insert element was evaluated by PCR followed by sequencing. [5]

Molecular typing

Genetic relationship was determined by pulsed-field gel electrophoresis (PFGE). The restriction patterns were analyzed using the BioNumerics software v. 3.0 (Applied Maths, Sint-Martens-Latem, Belgium). Percentage similarity between firgerprints was scored by the Dice coefficient. [6] Sequence Typing (ST) was performed by Multilocus Sequence Typing (MLST). DNA sequences were compared with MLST page Institut Pasteur, France to obtain the corresponding alleles and the ST number.

Statistical analysis

All clinical data were entered into a Research Electronic Data Capture (Redcap) database and SAS v.9.2 (SAS Institute, Cary, NC, USA), and analysed by univariate and multivariate models. Dichotomized and categorical data were analysed with the chi-squared test or Fisher's exact test. For continuous variables, the t-test or analysis of variance (ANOVA) was used. Bivariate analyses were performed to verify the associations between dependent and independent variables, and those achieving a pre-specified level of significance ($P < 0.05$) were included in the multivariable analysis.

RESULTS AND DISCUSSION

A total of 21 CRAB were isolated from colonization sites. The newborns hospitalized were admitted in NICU and NIU with ages ranging from 2 to 13 days and remaining hospitalized from 6 to 61 days following admission. All newborns were considered premature (<37 weeks) and fifteen had low birthweight (<2500g) (Table I). In this study, newborns colonized by CRAB were exposed to previous use of extended-spectrum beta-lactam (100%), aminoglycosides (83.3%) and cephalosporins (72.2%). These results are in agreement with previously reported findings that showed prior antimicrobial exposure contributed to the dissemination of carbapenem-resistant isolates in hospitalized patients. [7]

In the univariate analysis, CRAB colonization was associated with respiratory syndromes, prematurity, peripheral access, previous exposure to antibiotics, use of beta-lactam and cephalosporins. There is a strong relationship between the variables, since all newborns were premature and used beta-lactam and peripheral access. Thus, no statistically significant results were observed in the multivariate analysis. During hospitalization, four patients who are colonized developed infection, however with the treatment they were recovery (Table 1). The analysis of data outcomes showed that 19.1% (n = 4) and 4.7% (n=1) of cases and controls died, respectively. The cause of death in cases could not be attributed to CRAB, but might be related to unfavourable clinical conditions, such as gastroschisis, neonatal anoxia, respiratory complications, congenital syphilis and heart diseases.

All *A. baumannii* were resistant to imipenem (MIC50 $\geq$ 8 mg/L^{-1}), meropenem (MIC50 $\geq$ 8 mg / L^{-1}), ceftazidime (MIC50 $\geq$ 32 mg / L^{-1}), ceftriaxone (MIC50 $\geq$ 32 mg / L^{-1}), cefepime (MIC50 $\geq$ 16 mg / L^{-1}), gentamicin (MIC50 $\geq$ 16 mg / L^{-1}), ciprofloxacin (MIC50 $\geq$ 4 mg / L^{-1}), ampicillin/sulbactam (MIC50 $\geq$ 16 mg / L^{-1}), piperacillin/tazobactam (MIC50 $\geq$ 128 mg / L^{-1}), (n =7) amikacin (MIC50 $\geq$ 32 mg / L^{-1}) and (n = 16) tigecycline (MIC50 $\geq$ 8 mg / L^{-1}). The CRAB were susceptible only to colistin ($MIC_{50} \leq$ 2 mg/L^{-1}), (n = 11) to

amikacin ($MIC_{50} \leq 16$ mg/L^{-1}) and (n = 2) tigecycline ($MIC_{50} \leq 2$ mg/L^{-1}). All strains were identified as carbapenemase producers by MALDI-TOF MS and sequencing confirmed the presence of IS*Aba1* upstream *bla*$_{OXA-23}$ and *bla*$_{OXA-51}$ genes. Thus, increased levels of resistance to carbapenems were very likely caused by IS*Aba1* upstream *bla*$_{OXA-23}$ genes. [2]

PFGE analysis identified that 90.47% of isolates (n = 19) with 100% similarity (cluster C) (Figure 1) were colonizing newborns admitted to NICU and NIU. Newborns hospitalized in NICU are transferred to NIU to continue treatment when clinical conditions improve. Thus, contact between patients, hands or contaminated medical equipment may have contributed to the dissemination of the clonal isolate. The impossibility of identifying a common source in an environmental reservoir is the major limitation of this study.

MLST typing showed the most prevalent ST1 associated with the international clone I. In Latin America, OXA-23-producing *A. baumannii* strains have also been reported as belonging to ST1, [8] ST25 [9] and ST79. [8, 10] Interestingly, in our study, only one isolate was closely related to ST79 and ST25. The description of the ST25 in this hospital can become emerging in our state, since this ST was reported in Bolivia, [9] a country that borders the state of Mato Grosso do Sul, which until the moment had not been described the presence of ST25.

Following the initial detection of this outbreak for CRAB, infection control measures have been implemented, as surveillance cultures from all neonates hospitalized for more than 48 hours in different wards or health institutions; single use of medical equipment; isolation of patients who had a positive culture for CRAB; environmental cleaning of all surfaces including walls, floors, ceilings, windows, furniture and medical equipment. After the implementation of these measures, there was a reduction in the incidence of *A. baumannii* with this resistance profile. In summary, these results highlight the importance of the active search for CRAB in newborns and importance of infection control measures to prevent transmission of clones among patients.

FINANCIAL SUPPORT

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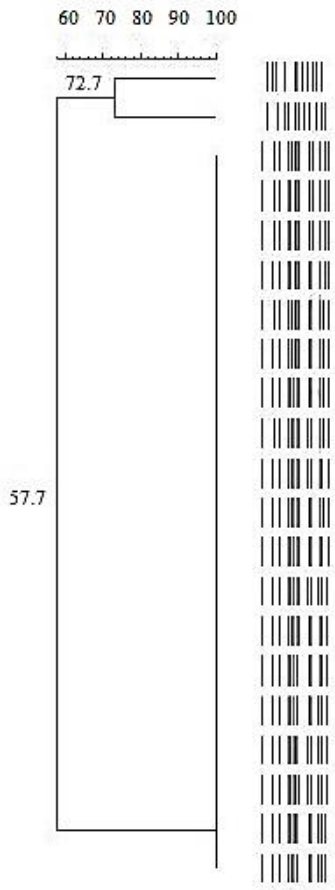
Table I. Clinical characteristics of the newborns colonization caused by carbapenem-resistant *Acinetobacter baumannii* isolates.

Patient identification	Gestational age, sex[a]	Birth weight[b]	Clinical isolates[c]	Date of admission	Date of isolation	Hospital unit	Length of stay[d]	Place prior to Admission	Type birth	Treatment	Outcome
1*	35, M	1850	Swab	09/17/2013	09/23/2013	NICU	21	OC	C	AMI(18mg), CEP(50mg), PNC(25mg)/13	Recovery
2	32, W	1082	Swab	09/14/2013	09/23/2013	NIU	54	OC	N	AMI(18mg), PNC(25mg)/11	Recovery
3	34, M	1608	Swab	09/23/2013	09/30/2013	NICU	41	OC	N	CAR(20mg), CEP(50mg), PNC(25mg)/20	Death
4*	27, W	1036	Swab	10/13/2013	10/21/2013	NICU	12	OC	N	AMI(15mg), PNC(25000UI)/1	Death
5	32, W	1780	Catheter tip	10/13/2013	10/21/2013	NICU	28	OC	C	AMI(15mg), PNC(25mg)/10	Recovery
6	31, W	1664	Swab	11/09/2013	11/11/2013	NIU	17	OC	N	CAR(40mg), CEP(50mg), PNC(25mg)/13	Recovery
7	31, W	1470	Swab	11/07/2013	11/12/2013	NICU	26	OC	N	AMI(18mg), PNC(25mg)/9	Recovery
8*	31, M	1790	Swab	11/07/2013	11/12/2013	NICU	19	OC	C	AMI(18mg), CEP(50mg), PNC(25mg)/12	Recovery
9	35, M	2630	Swab	11/09/2013	11/12/2013	NIU	6	AH	N	AMI(18mg), PNC(25mg)/5	Recovery
10	33, M	2608	Swab	11/09/2013	11/12/2013	NIU	7	OC	N	AMI(18mg), PNC(25mg)/8	Recovery
11*	31, W	698	Swab	11/13/2013	11/19/2013	NICU	61	OC	C	AMI(18mg), CAR(20mg), CEP(50mg), PNC(25mg)/18	Recovery
12	29, W	1294	Swab	11/15/2013	11/19/2013	NICU	59	OC	C	AMI(18mg), CAR(20mg), CEP(50mg), PNC(25mg)/15	Death
13	29, M	1320	Swab	11/15/2013	11/19/2013	NICU	59	OC	C	AMI(18mg), CEP(50mg), PNC(25mg)/11	Recovery
14	36, M	3192	Catheter tip	12/04/2013	12/16/2013	NICU	16	AH	C	CEP(50mg), PNC(25mg)/10	Recovery
15	36, W	3120	Swab	01/04/2014	01/07/2014	NIU	9	OC	C	CEP(50mg), PNC(25mg)/9	Recovery
16	31, W	1650	Swab	01/06/2014	01/11/2014	NIU	26	OC	C	AMI(18mg), PNC(25mg)/7	Death
17	32, M	1830	Swab	01/08/2014	01/15/2014	NIU	23	AH	N	CEP(50mg), PNC(25mg)/8	Recovery
18	33, M	1990	Swab	01/06/2014	01/16/2014	NICU	49	OC	C	AMI(18mg), CAR(20mg), CEP(50mg), PNC(25mg)/21	Recovery
19	30, M	1180	Catheter tip	01/29/2014	02/04/2014	NIU	52	AH	N	AMI(18mg), CEP(50mg), PNC(25mg)/19	Recovery
20	34, F	964	Swab	01/25/2014	02/05/2014	NIU	47	OC	C	AMI(18mg), CAR(20mg), CEP(50mg), PNC(25mg)/18	Recovery
21	32, M	1724	Swab	04/20/2014	04/26/2014	NIU	10	OC	N	AMI(18mg), CEP(50mg), PNC(25mg)/8	Recovery

[a]Age in days. [b]Birth weight in grams. [c]Rectal swab. [d]Length of stay in days. [e]Treatment (dosage and days of therapy). W, woman; M, man. NICU, Neonatal Intensive Care Unit; NIU, Neonatal Intermediate Unit. OC, Obstetric Center; AH, Another Hospital. C, Cesarean; N, Normal. *Patients who developed infection during hospitalization. AMI, Aminoglycoside; CAR, Carbapenems; CEP, Cephalosporins; PNC, Penicill.



Strain	Ward	Outcome	Cluster	MLST
123	NIU	Recovery	A	ST79
103	NIU	Recovery	B	ST25
8	NICU	Recovery	C	ST1
2	NIU	Recovery	C	ST1
9	NICU	Death	C	ST1
15	NICU	Death	C	ST1
16	NICU	Recovery	C	ST1
39	NIU	Recovery	C	ST1
37	NICU	Recovery	C	ST1
42	NICU	Recovery	C	ST1
46	NIU	Recovery	C	ST1
55	NIU	Recovery	C	ST1
35	NICU	Recovery	C	ST1
53	NICU	Death	C	ST1
38	NICU	Recovery	C	ST1
65	NICU	Recovery	C	ST1
74	NIU	Recovery	C	ST1
73	NIU	Death	C	ST1
64	NIU	Recovery	C	ST1
62	NICU	Recovery	C	ST1
58	NIU	Recovery	C	ST1

Figure 1. Dendrogram displaying the genetic relatedness of 21 CRAB isolated from newborns during an outbreak in NICU and NIU. The 19 isolates containing 100% similarity are grouped in cluster C (PFGE) and ST1 (MLST).

Supplementary material

Table II. Factors associated with carbapenem-resistant *Acinetobacter baumannii* isolates.

Risk factor	Case patients (n = 21)	Control patients (n = 21)	Univariate analysis OR (95% CI)	P
Age (days)	1.00±0	1.85±1.93		
Neonate weight				
AGA	11 (52.38)	13 (61.9)	1.47 (0.43-5.04)	0.53
SGA	10 (47.62)	8 (38.09)	1.47 (0.43-5.04)	0.53
Comorbidities				
Congenic cardiopathies	7 (33.33)	3 (14.29)	3 (0.65-13.74)	0.15
Icterus	6 (28.57)	3 (14.29)	2.4 (0.51-11.26)	0.26
Respiratory syndromes	13 (61.9)	6 (28.57)	4.06 (1.11-14.80)	**0.03**
Hospitalization				
Low weight	17 (80.95)	13 (61.90)	2.61 (0.64-10.61)	0.17
Prematurity (GA<37 weeks)	21 (100)	12 (57.14)	32.68 (1.74-610)	**<0.01**
Gemini	4 (19.05)	4 (19.05)	1 (0.21-4.66)	1.00
Previous hospitalization	4 (19.05)	9 (42.86)	0.31 (0.07-1.26)	0.09
Surgical procedure	1 (4.76)	2 (9.52)	0.47 (0.03-5.67)	0.55
Use of antimicrobials				
Previous exposure	21 (100)	17 (80.95)	11.05 (0.55-219.68)	**0.03**
Aminoglycosides	17 (80.95)	12 (57.14)	3.18 (0.79-12.80)	0.09
Beta-lactam	21 (100)	16 (76.19)	14.33 (0.73-278.06)	**0.02**
Carbapenens	7 (33.33)	6 (28.57)	1.25 (0.33-4.63)	0.74
Cephalosporins	14 (66.67)	7 (33.33)	4 (1.10-14.43)	**0.03**
Presence of device				
Peripheral access	21 (100)	14 (66.67)	0.66 (0.49-0.90)	**0.01**
ETT	15 (71.42)	9 (42.86)	3.33 (0.92-12.01)	0.06
CVA	7 (33.33)	4 (19.04)	2.12 (0.51-8.77)	0.29
Probes	2 (9.52)	1 (4.76)	2.10 (0.17-25.17)	0.55

OR – Odds ratio; CI – Confidence interval. In bold, variables with level of significance ($P < 0.05$).

GA, gestational age; ETT, endotracheal tube; CVA, central venous access.

8 Manuscrito em elaboração

High mortality rate associated with OXA-23-producing *Acinetobacter baumannii*: a case-control study

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ABSTRACT

Acinetobacter baumannii are opportunistic pathogens related to important cases of nosocomial infections. Risk factors associated with OXA-23-producing *A. baumannii* strains were investigated in this case-control study from September 2013 to September 2015. Bacterial identification and antimicrobial susceptibility were performed by Vitek®2 and confirmed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). The presence of β-lactamase genes was analyzed by polymerase chain reaction (PCR) and sequencing. Genetic relatedness among strains was determined by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). This study included 82 patients. A nasogastric tube, hemodialysis and use of cephalosporins were associated with OXA-23-producing *A. baumannii*. Forty-one OXA-23-producing *A. baumannii* strains were isolated from adult patients. These strains showed sensitivity only to amikacin, gentamicin, tigecycline and colistin and presented IS*Aba1* upstream $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes. Twelve-six OXA-23-producing *A. baumannii* strains were clonally related and patients infected with the genetic profile ST79 had a higher mortality rate (34.6%). OXA-23-producing *A. baumannii* strains have been associated with several healthcare-related risk factors and high mortality rates in ICUs.

Keywords: Oxacillinase, multi-drug resistant, ICU.

INTRODUCTION

The emergence of carbapenem-resistant *Acinetobacter baumannii* has been reported in many countries and has become a matter of great concern. [1] They cause numerous diseases, are hard to treat and have the potential to spread within health care facilities. Infections with these organisms are associated with high rates of morbidity and mortality. [2]

The dissemination of *A. baumannii* that harbor carbapenemase-encoding genes pose a significant threat to the control and treatment management of nosocomial infections and have been associated with hospital outbreaks in various geographic regions. [3] In addition, the rates of carbapenem resistance among *A. baumannii* have dramatically increased. Among the resistance mechanisms, production of carbapenemase OXA-23, OXA-24, OXA-51 and OXA-58 are the more prevalent in *A. baumannii* strains, associated with genetic elements, such as insert sequences, integrons, transposons and plasmids. [4, 5] Several outbreaks of carbapenem-resistant *A. baumannii* bacteria have occurred, [1, 6] making them an international clinical and public health concern. [3]

In Brazil, the first description of OXA-23-producing *A. baumannii* occurred in the Southern region of the country in 2003,[7] in addiction, high rates of resistance to carbapenems have been reported in Brazilian hospitals related to the clonal dissemination of OXA-23-producing *A. baumannii*, becoming a problem mainly in ICUs. [8, 9] The presence of IS*Aba1* upstream $bla_{\text{OXA-23}}$ is associated with increased expression of resistance to carbapenems, which has made it even more difficult to control the spread of this microorganism in hospital environments. [9]

Although many studies have reported on the drug resistance profile of *A. baumannii* worldwide, [1] there is limited information regarding the epidemiology of carbapenem-resistant *A. baumannii* in Brazil. A case-control and molecular study were performed in a public hospital in a Central-West Brazilian state, to identify the risk factors associated with OXA-23-producing *A. baumannii* strains in an Adult Intensive Care Unit (ICU). Understanding the risk factors associated with carbapenem-resistant *A. baumannii* in healthcare facilities may be important for targeting interventions and reducing hospital transmission.

MATERIAL AND METHODS

Case-control study

To identify risk factors, a case-control study was conducted in the an adult ICU. Patients hospitalized between September 2013 and September 2015 in a public hospital located in the city of Dourados, Mato Grosso do Sul (a Central-West Brazilian state), were included in this study. A case was defined as a patient infected or colonized by OXA-23-producing *A. baumannii* strains and control were patients without the isolation of *A. baumannii* in the first 48 hours after admission. [6] For each case, one control was selected from patients admitted within the study period matched by age, clinical manifestation and hospital ward. All medical, nursing and microbiological records of patients hospitalized were reviewed. Clinical records from inpatients were reviewed and the following data were recorded: demographics; medical history and co-morbid conditions; location prior to admission; ward of admission; hospital course (duration and ward location); invasive procedures (device use and surgery) receiving of mechanical ventilation; treatment with immunosuppressant drugs; antibiotic exposure history; source of infection (blood, urinary tract, wound, respiratory source or other) and outcome (recovery/death).

The comorbidities included diabetes mellitus, cardiovascular disease, renal failure, respiratory failure, chronic obstructive pulmonary disease, alcoholism, tabagism, neoplasia, neurological disease, sepsis, substance abuse, HIV infection, decubitus ulcers, active cancer and hypertension were evaluated. Both the individual and cumulative numbers of co-morbid conditions were evaluated. All antibiotics administered for $\geq$ 24 hours during the current hospitalization were recorded. The information collected included the drug name, start date, dose, route of administration, dosing frequency and total duration of use. Both individual and cumulative antibiotic exposures were evaluated.

Bacterial isolates

The OXA-23-producing *A. baumannii* strains during the study period were obtained from 41 adults patients, collected on different days and infection sites. Colonization was defined as the isolation of strains without clinical manifestation of infection. Clinical infection was defined by medical diagnosis according to clinical criteria (sepsis, fever, changes in frequency or color of secretions, or new radiological findings) associated with the decision to initiate antibiotic therapy, as well as isolation of one OXA-23-producing *A. baumannii* strains. [10] The study was conducted with the approval of the Research Ethics Committee from the Universidade Federal da Grande Dourados (no. 877.292/2014).

Bacterial identification, susceptibility testing and phenotypic assays

Bacterial species were identified using the Vitek®2 (bioMérieux, Hazelwood, MO) and confirmed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS), using a Microflex LT spectrometer (Bruker Daltonics, MA, USA), as previously described. [11] The minimal inhibitory concentrations (MICs) of antimicrobials was determined by Vitek®2 for imipenem, meropenem, ceftazidime, ceftriaxone, cefepime, gentamicin, ciprofloxacin, colistin, tigecycline, ampicillin/sulbactam and piperacillin/tazobactam. Susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI/2016) guidelines. [12] As for tigecycline it was interpreted by the European Committee on Antimicrobial Susceptibility Testing (EUCAST/2016) guidelines. [13] Preliminary screening for the presence of carbapenemases was performed by ertapenem (2 and 4 hours) hydrolysis using MALDI-TOF MS. [14] Carbapenem hydrolysis was considered positive if the ertapenem intact-molecule mass peak (475 m/z) and that of its monosodium salt (497 m/z) disappeared completely. [14, 15]

PCR amplification

The presence of β-lactamase genes (*bla*$_{IMP-1}$, *bla*$_{NDM-1}$, *bla*$_{VIM-1}$, *bla*$_{KPC-2}$, *bla*$_{OXA-23}$, *bla*$_{OXA-24}$, *bla*$_{OXA-48}$, *bla*$_{OXA-51}$, *bla*$_{OXA-58}$) [16, 17] and IS*Aba1* insert element [9] was evaluated by polymerase chain reaction (PCR) and followed by sequencing using specific primers, as previously described. [18] The DNA sequences and their derived protein sequences were analyzed using the Lasergene Software Package (DNASTAR) and compared with the sequences deposited in GenBank.

Molecular typing by pulsed-field gel electrophoresis (PFGE)

The genetic relationship among the OXA-23-producing *A. baumannii* strains was determined by PFGE. Genomic DNA was digested using the restriction enzyme *Apa*I (New England BioLabs, Ipswich, MA, USA). The restriction patterns were analyzed using the BioNumerics software v. 3.0 (Applied Maths, Sint-Martens-Latem, Belgium). Percentage similarity between firgerprints was scored by the Dice coefficient. [19] The unweighted pair group method with arithmetic mean and a 1.5% tolerance limit was used to reconstruct a dendrogram and cluster analyses were performed using the algorithms available within the BioNumerics software package v. 6.0 (Applied Maths, Sint-Martens-Latem, Belgium).

Multilocus Sequence Typing (MLST)

To determine the Sequence Typing (ST) of 41 OXA-23-producing *A. baumannii* strains, MLST technique was performed. Each sample was subjected to amplification and partial sequencing of the seven standard genes for the genus *Acinetobacter* spp. (*cpn*60, *fus*A, *glt*A, *pyr*G, *rec*A, *rpl*B and *rpo*B) using primers as described previously. [20] DNA sequences obtained were analyzed using the program Lasergene Software Package (DNAStar, Madison, USA) and then the sequences were deposited together by isolated evaluated directly in the

MLST page Institut Pasteur, France in order to obtain the corresponding alleles and consequently, the ST number. [20, 21]

Statistical analysis

All clinical data were deposited in the Research Electronic Data Capture (Redcap) database and the statistical analysis was performed by SAS v. 9.2 (SAS Institute), using the univariate and multivariate models. Dichotomized and categorical data were analyzed with the chi-square test or Fisher´s exact test. For continuous variables, the *t*-test or ANOVA was used. Bivariate analyses were performed to verify the associations between the dependent and independent variables, and those achieving a pre-specified level of significance ($P<0.05$). Logistic regression analysis was used to estimate the crude and adjusted odds ratios.

RESULTS

Patients of the study

The patients were hospitalized in ICUs and their ages ranged from 24–87 years and 58.5% (n = 24) were men. Prior to isolation of OXA-23-producing *A. baumannii,* all patients had received antimicrobial regimens, which included penicillin, third or fourth generation cephalosporins, aminoglycosides, fluoroquinolones, amikacin, tigecycline, piperacillin-tazobactam, trimethoprim, carbapenems and polymyxins. A total of 41 OXA-23-producing *A. baumannii* strains were recovered from 41 patients between 1 and 95 days following admission. Out of these, 92.6% (n = 38) had a history of the previous hospitalization in the same facilities or in other hospitals. The remaining patients had no history of previous hospitalization and were admitted directly into the ICU from the emergency room.

Among 41 OXA-23-producing *A. baumannii* strains, 26.8% (n=11) were from colonization sites and 73.2% (n=30) were obtained from infection. Out of the identified

174 strains, 56.1% (n = 23) were isolated from tracheal aspirates, 26.8% (n = 11) swabs, 7.3% (n
175 = 3) from wounds, 4.9% (n = 2) blood and 4.9% (n = 2) urine (Figure 1). The case-control
176 study was performed in 82 patients (41 cases and 41 controls) and there were no significant
177 differences ($P > 0.05$) among cases and controls in regards to baseline demographics. In the
178 multivariable analysis, OXA-23-producing *A. baumannii* strains were associated with
179 nasogastric tubes, hemodialysis and use of cephalosporins (Table 1). The analysis of data on
180 patient outcomes revealed that OXA-23-producing *A. baumannii* patients had a higher
181 mortality rate compared to controls, being 34.1% (n=14) and 24.3% (n=10), respectively, ($P =$
182 0.5). The other deaths were due to renal, cardiovascular, pulmonary, neoplastic and cancer
183 diseases.
184
185 Susceptibility testing
186 All 41 (100%) OXA-23-producing *A. baumannii* strains were resistant to imipenem
187 ($MIC_{50} \geq 8$ mg/L^{-1}), meropenem ($MIC_{50} \geq 8$ mg/L^{-1}), ceftazidime ($MIC_{50} \geq 32$ mg/L^{-1}),
188 ceftriaxone ($MIC_{50} \geq 32$ mg/L^{-1}), cefepime ($MIC_{50} \geq 16$ mg/L^{-1}), gentamicin ($MIC_{50} \geq 16$
189 mg/L^{-1}), ciprofloxacin ($MIC_{50} \geq 4$ mg/L^{-1}), ampicillin/sulbactam ($MIC_{50} \geq 16$ mg/L^{-1}),
190 piperacillin/tazobactam ($MIC_{50} \geq 128$ mg/L^{-1}), of 41 strains 90.2% (n = 37) were resistant to
191 gentamicin ($MIC_{50} \geq 8$ mg/L^{-1}), 43.9% (n = 18) to tigecycline ($MIC_{50} \geq 4$ mg/L^{-1}) and 34.1%
192 (n = 14) to amikacin ($MIC_{50} \geq 32$ mg/L^{-1}). All strains showed sensibility to colistin ($MIC_{50} \leq$
193 2 mg/L^{-1}), of 41 strains 65.9% (n = 27) amikacin ($MIC_{50} \leq 16$ mg/L^{-1}), 56.1% (n =23)
194 tigecycline ($MIC_{50} \leq 2$ mg/L^{-1}) and 9.8% (n = 4) gentamicin ($MIC_{50} \leq 4$ mg/L^{-1}).
195
196 Molecular testing, PFGE and MLST analysis
197 The 41 OXA-23-producing *A. baumannii* strains were classified as carbapenemase
198 producers by MALDI-TOF MS and presented hydrolysis after 4 hours of incubation. PCR

amplification and sequencing showed that the IS*Aba1* upstream $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ genes were present in all carbapenem-resistant strains. The presence of $bla_{\text{IMP-1}}$, $bla_{\text{NDM-1}}$, $bla_{\text{VIM-1}}$, $bla_{\text{KPC-2}}$, $bla_{\text{OXA-24}}$, $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-58}}$ genes was not detected. PFGE analysis of 41 OXA-23-producing *A. baumannii* strains identified 46.3% (n = 19) with more than 80.06% similarity, as shown in the dendrogram (Fig. 1, cluster E). ST79 was the genetic profile predominant with 63.4% (n = 26) strains in MLST (Fig. 1, clusters E and F). Out of the 26 strains, 84.6% (n = 22) were isolated from infections sites. Analysis of the data revealed that patients infected or colonized with this predominant clonal type had a higher mortality rate, being 34.6% (n=9) ($P \leq 0.01$) compared to patients infected with other strains.

DISCUSSION

OXA-23-producing *A. baumannii* strains have been increasingly reported worldwide, [8, 22] affecting severely ill patients. In addition, this pathogen is associated with high morbidity and mortality rates. [8] Infected and colonized patients represent reservoirs for horizontal transmission and spread of multidrug resistance *A. baumannii*, especially in ICUs. [23] During the study period, 30 patients were infected and 11 colonized by OXA-23 *A. baumannii* in the adult ICU, once high rates of infection and colonization caused by *A. baumannii* are reported in ICUs. [9]

Among the patients, 46.3% (n = 19) have age > 60 years, presented multiple comorbidities as diabetes, hypertension, chronic diseases and sepsis (Table 1), besides they were submitted to invasive procedures as mechanical ventilation, previous surgery, use of central venous catheter, use of urinary catheter and nasogastric tube (Table 1) and 65.8% (n = 27) patients have prolonged hospitalization time (>15 days). All patients were exposed to antimicrobial treatments included penicillin, third or fourth generation cephalosporins, aminoglycosides, fluoroquinolones, amikacin, tigecycline, piperacillin-tazobactam,

trimethoprim, carbapenems and polymyxins, however, 78% (n = 32) patients had antimicrobial treatment >7 days. In our study, 92.6% (n = 38) patients had a history of the previous hospitalization, however, this factor was not statistical. These data reinforce the importance of factors such as age, comorbidities, invasive procedures, prolonged hospitalization and prolonged exposure to various antimicrobial classes may have influenced the rates of resistance and dissemination of OXA-23-producing *A. baumannii* in the adult UCI, as already reported in other studies.[3, 24, 25] Once that these factors in hospitalized patients contributed to the dissemination of multidrug-resistant strains [10] and constitute a therapeutic problem, affecting the clinical outcome.[8]

Assessment of the factors that predict carbapenem resistance by multivariable analysis demonstrated that nasogastric tube, hemodialysis and cephalosporins users were associated with OXA-23-producing *A. baumannii*. These risk factors may be related to the ability of *A. baumannii* colonize various surfaces and medical devices, with the conditions of patients as comorbidity and invasive procedures, as well as the use of broad spectrum antibiotics. [26]

The nasogastric tube has been described like as a risk factor for acquiring carbapenem resistant *A. baumannii* in hospitalized patients [27, 28] and hemodialysis for the acquisition of imipenem resistant *A. baumannii* infections. [29] Antibiotic exposures are frequently reported risk factor for multidrug resistance *A. baumannii*. [3] In our study, 82.9% (n = 34) of the patients had exposure to carbapenems, however, the use cephalosporins increased the risk factor to had OXA-23-producing *A. baumannii*. [30]

The carbapenem resistance may occur due to the decrease in membrane permeability, the presence of efflux pumps, changes in the antibiotic binding site. [2] However, carbapenem-hydrolysing class D β-lactamases (CHDLs) as OXA-23, OXA-24, OXA-51 and OXA-58 are the main mechanism of carbapenem resistance in *A. baumannii*. [8, 22] In our study, all carbapenem-resistant *A. baumannii* strains showed $bla_{\text{OXA-23}}$ gene and $bla_{\text{OXA-51}}$ and IS*Aba1*

247 insertion sequence upstream *bla*$_{\text{OXA-23}}$. This insertion sequence is considered a strong
248 promoter and cloud been increased resistance levels of carbapenems.[2, 8, 22]

249 PFGE analysis of 41 OXA-23-producing *A. baumannii* identified 7 clusters (A-G),
250 however, 46.3% (n=19) with more than 80.06% similarity (cluster E) was predominant in this
251 study. MLST typing showed that ST1 (CC1), [8, 9] ST15 (CC15), ST25 (CC25) [8, 22] and ST79
252 (CC79), [8, 9] were the predominant genotypes identified in this study. The ST1 correspond to
253 international clone I and along with the ST15 and ST79 are the genotypes of OXA-23-
254 producing *A. baumannii* most reported in Brazilian hospitals, [8, 9] and ST25 has already been
255 reported in Brazilian regions such as Midwest, Southeast and North. [8, 9] The description of the
256 ST25 could be associated with the boundary of the Mato Grosso do Sul state makes the
257 Bolivia, where it *A. baumannii* strains presenting this genotype were reported. [22] In the study,
258 the presence of ST25 was associated with 100% morbidity of patients.

259 The patients infected or colonized with predominant clonal type ST79 had a higher
260 mortality rate 34.6% ($P \leq 0.01$) caused by OXA-23-producing *A. baumannii* compared to
261 patients infected with other genotypes of *A. baumannii*, which reinforces the clonal
262 dissemination of OXA-23-producing *A. baumannii* strains in the ICUs. [9] Furthermore, the
263 MLST has different genetic patterns, which demonstrates the easily of *A. baumannii* acquire
264 resistance and shows the importance of having effective control measures to restrict its spread
265 in nosocomial environments. [8]

266 Our findings showed that the acquisition of OXA-23-producing *A. baumannii* was
267 associated with several healthcare related risk factors and high mortality rates in ICUs. In
268 addition, the higher mortality rate of patients was caused by the ST79 genotype. With the
269 emergence of infection and colonization by OXA-23-producing *A. baumannii*, these results
270 show the importance of reinforcing hospital infection control measures, particularly with

patients transferred from other hospitals.Thus the spread of OXA-23-producing *A. baumannii* strains with the profiles presented in our study do not emerge.

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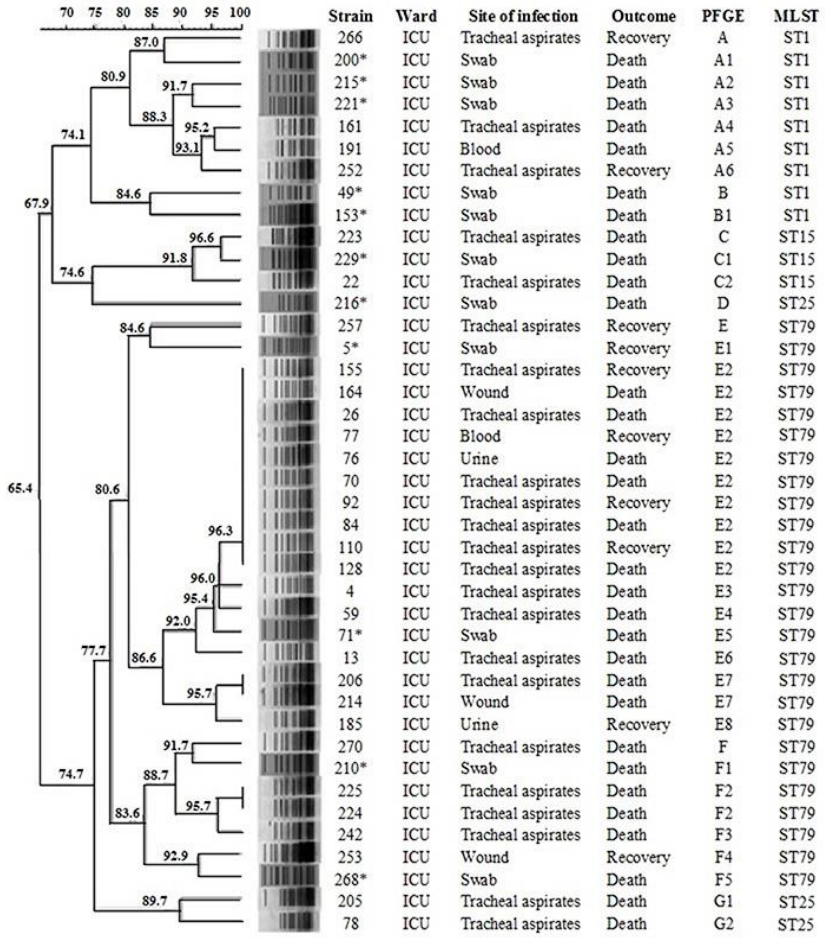
377 antibiotic exposure on occurrence of nosocomial carbapenem-resistant *Acinetobacter*
378 *baumannii* infection: A case control study. Journal Infect Chemotherapy. 2014, 1-6.

379 Table 1. Summary of risk factors associated with OXA-23-producing *Acinetobacter baumannii*.

Variables	Case patients (n = 41)	Control patients (n = 41)	Univariable analysis		Multivariable analysis	
			OR (95% CI)	P	OR (95% CI)	P
Age (years)	63. 84±15.42	61.74±15.14				
Comorbidities						
Diabetes	16 (39.02)	8 (19.51)	2.64 (0.97-7.14)	0.05		
Alcoholism	8 (19.51)	6 (14.63)	1.41 (0.44-4.51)	0.55		
Hypertension	24 (58.53)	21 (51.22)	1.3 (0.56-3.21)	0.50		
Ulcers	6 (14.63)	3 (7.32)	2.17 (0.50-9.35)	0.28		
Pulmonary disease	2 (4.88)	8 (19.51)	0.21 (0.04-1.06)	0.04		
Chronic heart failure	6 (14.63)	8 (19.51)	0.70 (0.22-2.25)	0.55		
Chronic renal failure	14 (34.15)	8 (19.51)	2.13 (0.78-5.85)	0.13		
Chronic respiratory failure	12 (29.27)	16 (39.02)	0.64 (0.25-1.62)	0.35		
Cancer	5 (12.20)	3 (7.32)	1.75 (0.39-7.90)	0.45		
Substance misuse	10 (24.39)	10 (24.39)	1 (0.36-2.74)	1.00		
Neurological disease	12 (29.27)	5 (12.20)	2.97 (0.94-9.43)	0.05		
Neoplasias	8 (19.51)	3 (7.32)	3.07 (0.75-12.53)	0.10		
HIV infection	2 (4.88)	1 (2.43)	2.05 (0.17-23.54)	0.55		
Sepsis	23 (56.10)	12 (29.27)	3.08 (1.23-7.69)	0.01		
Risk factor						
Mechanical ventilation	33 (80.49)	32 (78.05)	1.16 (0.39-3.38)	0.78		
Previous surgery	28 (68.29)	19 (46.34)	2.49 (1.01-6.13)	0.04		
Central venous catheter	29 (70.73)	27 (65.85)	1.25 (0.49-3.18)	0.63		
Urinary catheter	28 (68.29)	29 (70.73)	0.89 (0.34-2.28)	0.81		
Use of imunosuppressive	5 (12.19)	1 (2.43)	5.55 (0.61-49.82)	0.08		
Hemodialisysis	10 (24.39)	3 (7.32)	4.08 (1.03-16.15)	0.03	4.87 (1.07-22.05)	0.03
Nasogastric tube	23 (56.10)	10 (24.39)	3.96 (1.54-10.16)	0.00	4.66 (1.59-13.66)	<0.01
Chest drainage	7 (17.07)	5 (12.20)	1.48 (0.42-5.12)	0.53		
Previous hospital admission	38 (92.68)	34 (82.93)	2.60 (0.62-10.89)	0.17		
Use of antimicrobials						
Previous exposure	41 (100)	39 (95.12)	5.25 (0.24-112.87)	0.15		
Aminoglicosydes	27 (65.85)	20 (48.78)	2.02 (0.83-4.92)	0.11		
Beta-lactam	20 (48.78)	12 (29.27)	2.30 (0.92-5.71)	0.07		
Carbapenens	34 (82.93)	31 (75.61)	1.56 (0.53-4.62)	0.41		
Cephalosporins	10 (24.39)	26 (63.41)	0.18 (0.07-0.48)	0.00	6.01 (2.04-17.69)	<0.01
Polymyxins	18 (43.90)	11 (26.83)	2.13 (0.84-5.38)	0.10		
Quinolones	5 (12.20)	9 (21.95)	0.49 (0.14-1.62)	0.24		

380 OR – Odds ratio; CI – Confidence interval. HIV, Human immunodeficiency virus.



Strain	Ward	Site of infection	Outcome	PFGE	MLST
266	ICU	Tracheal aspirates	Recovery	A	ST1
200*	ICU	Swab	Death	A1	ST1
215*	ICU	Swab	Death	A2	ST1
221*	ICU	Swab	Death	A3	ST1
161	ICU	Tracheal aspirates	Death	A4	ST1
191	ICU	Blood	Death	A5	ST1
252	ICU	Tracheal aspirates	Recovery	A6	ST1
49*	ICU	Swab	Death	B	ST1
153*	ICU	Swab	Death	B1	ST1
223	ICU	Tracheal aspirates	Death	C	ST15
229*	ICU	Swab	Death	C1	ST15
22	ICU	Tracheal aspirates	Death	C2	ST15
216*	ICU	Swab	Death	D	ST25
257	ICU	Tracheal aspirates	Recovery	E	ST79
5*	ICU	Swab	Recovery	E1	ST79
155	ICU	Tracheal aspirates	Recovery	E2	ST79
164	ICU	Wound	Death	E2	ST79
26	ICU	Tracheal aspirates	Death	E2	ST79
77	ICU	Blood	Recovery	E2	ST79
76	ICU	Urine	Death	E2	ST79
70	ICU	Tracheal aspirates	Death	E2	ST79
92	ICU	Tracheal aspirates	Recovery	E2	ST79
84	ICU	Tracheal aspirates	Death	E2	ST79
110	ICU	Tracheal aspirates	Recovery	E2	ST79
128	ICU	Tracheal aspirates	Death	E2	ST79
4	ICU	Tracheal aspirates	Death	E3	ST79
59	ICU	Tracheal aspirates	Death	E4	ST79
71*	ICU	Swab	Death	E5	ST79
13	ICU	Tracheal aspirates	Death	E6	ST79
206	ICU	Tracheal aspirates	Death	E7	ST79
214	ICU	Wound	Death	E7	ST79
185	ICU	Urine	Recovery	E8	ST79
270	ICU	Tracheal aspirates	Death	F	ST79
210*	ICU	Swab	Death	F1	ST79
225	ICU	Tracheal aspirates	Death	F2	ST79
224	ICU	Tracheal aspirates	Death	F2	ST79
242	ICU	Tracheal aspirates	Death	F3	ST79
253	ICU	Wound	Recovery	F4	ST79
268*	ICU	Swab	Death	F5	ST79
205	ICU	Tracheal aspirates	Death	G1	ST25
78	ICU	Tracheal aspirates	Death	G2	ST25

Figure 1. Dendrogram displaying the genetic relatedness of 41 OXA-23-producing *A. baumannii* strains recovered in a Brazilian teaching hospital, based on PFGE data and MLST content. Asterisks indicate the colonizing strains. ICU, Intensive Care Unit.

9 Considerações finais do estudo

Os resultados obtidos neste estudo indicam a disseminação de cepas de *A. baumannii* resistentes a carbapenêmicos isoladas de pacientes internados em UTIs Neonatal e Adulto de um hospital público de Dourados/MS. Os estudos de caso-controle realizados nestas UTIs demonstraram que a colonização por *A. baumannii* resistentes a carbapenêmicos em neonatos estava associada a síndromes respiratórias, prematuridade, exposição prévia a antibióticos, uso de betalactâmicos, uso de cefalosporinas e acesso periférico. Enquanto que na UTI adulto, outros fatores foram indicados como de risco para o desenvolvimento de infecções e colonizações, como o uso de tubo nasogástrico, hemodiálise e uso de cefalosporinas. A divergência entre os fatores encontrados nestas duas UTIs se dá pela diferença entre os dois grupos estudados, uma vez que neonatos apresentam sistema imunológico, comorbidades, tratamento e procedimentos invasivos bem distintos quando comparados com os pacientes adultos.

Durante o período do estudo, foi possível observar que as 59 cepas de *A. baumannii* apresentaram um índice de resistência alta aos antibióticos testados, bem como, todas as cepas apresentaram a sequência de inserção IS*Aba1* à montante do gene $bla_{\text{OXA-23}}$ e o gene $bla_{\text{OXA-51}}$. Foi observado um surto ocasionado por *A. baumannii* resistentes a carbapenêmicos na UTI neonatal, enquanto que na UTI adulto pode-se observar uma alta diversidade genética. O estudo da ancestralidade deste isolados identificou os genótipos ST1 (CC1), ST15 (CC15), ST25 e ST79 (CC79), correspondendo aos grupos clonais disseminados mundialmente. No entanto, a frequência do genótipo ST1 foi prevalente em ambas UTIs, além disso, na UTI adulto, foi observado que 34,6% (n=9) dos pacientes evoluíram ao óbito decorrente de sepse por *A. baumannii* pertencentes ao genótipo ST79.

A presença do ST25 no estado do Mato Grosso do Sul ainda não havia sido relatada na literatura, mas pode estar ocorrendo devido o estado fazer divisa com a Bolívia, país em que já foi descrita a presença deste genótipo. Estes dados indicam que a detecção precoce, bem como a elaboração de medidas de controle adequadas, são de extrema importância para a redução da infecção hospitalar.

Anexos

Anexo A. Parecer consubstanciado do Comitê de Ética em Pesquisa

FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Epidemiologia molecular de bactérias gram negativas produtoras de carbapenemases isoladas em Hospitais de Dourados-MS.

Pesquisador: Simone Simionatto

Área Temática: Área 3. Fármacos, medicamentos, vacinas e testes diagnósticos novos (fases I, II e III) ou não registrados no país (ainda que fase IV), ou quando a pesquisa for referente a seu uso com modalidades, indicações, doses ou vias de administração diferentes daquelas estabelecidas, incluindo seu emprego em combinações.

Versão: 4

CAAE: 05666812.3.0000.5160

Instituição Proponente: FUNDACAO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS

Patrocinador Principal: FUND. DE APOIO E DE DESENV. DO ENSINO,CIENCIA E TECN. DO ESTADO DO MS

DADOS DO PARECER

Número do Parecer: 877.292

Data da Relatoria: 09/09/2014

Apresentação do Projeto:

O presente projeto propõe realizar um estudo de epidemiologia molecular de cepas de Enterobactérias produtoras de KPC isoladas de pacientes atendidos no Hospital Universitário HU) da Universidade Federal da Grande Dourados (UFGD). Os resultados obtidos com as técnicas moleculares utilizadas para o diagnóstico e estudo de doenças infecciosas de origem hospitalar serão associados com a prevalência dos agentes envolvidos nestas enfermidades. Através da revisão de prontuários de pacientes internados no hospital será possível identificar os fatores de riscos associados à infecção ou colonização por microorganismos multirresistentes de interesse clínico. Também serão realizadas investigações sobre a relação entre a gravidade dos pacientes e a aquisição dos isolados resistentes, a influência do tempo de exposição ao ambiente hospitalar sobre a aquisição destes agentes infecciosos. Acredita-se que estes estudos possam contribuir para traçar medidas de contenção adequadas bem como para evitar futuros surtos de
infecção dentro do ambiente hospitalar, contribuindo desta forma com ações de vigilância em saúde e consequentemente reduzindo os gastos do Sistema Único de Saúde com internações provenientes destes problemas. ao ambiente hospitalar sobre a aquisição destes agentes

Endereço: Rua Melvin Jones, 940
Bairro: Jardim América **CEP:** 79.803-010
UF: MS **Município:** DOURADOS
Telefone: (67)3410-2853 **E-mail:** cep@ufgd.edu.br

FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

Continuação do Parecer: 877.292

infecciosos. Acredita-se que estes estudos possam contribuir para traçar medidas de contenção adequadas bem como para evitar futuros surtos de infecção dentro do ambiente hospitalar, contribuindo desta forma com ações de vigilância em saúde e consequentemente reduzindo os gastos do Sistema Único de Saúde com internações provenientes destes
problemas.ao ambiente hospitalar sobre a aquisição destes agentes infecciosos. Acredita-se que estes estudos possam contribuir para traçar medidas de contenção adequadas bem como para evitar futuros surtos de infecção dentro do ambiente hospitalar, contribuindo desta forma com ações de vigilância em saúde e consequentemente reduzindo os gastos do Sistema Único de Saúde com internações provenientes destes problemas.

Objetivo da Pesquisa:

Estudar a ocorrência de Enterobactérias produtoras de carbapenemase (KPC) isoladas de pacientes atendidos no Hospital Universitário de Dourados, visando identificar os fatores de riscos associados a aquisição de infecções causadas por estas bactérias.

Avaliação dos Riscos e Benefícios:

Quanto aos benefícios parece ser uma proposta que possibilitará auxiliar ações de vigilância em saúde. A avaliação dos riscos inerentes à coleta das amostras dos pacientes é inexistente. No entanto, a pesquisa é retrospectiva, uma vez que o material já foi coletado em procedimento padrão da instituição em que será realizada a pesquisa, o que torna suficiente a avaliação ora apresentada no protocolo.

Comentários e Considerações sobre a Pesquisa:

O tema é relevante e os resultados da pesquisa podem contribuir com ações de vigilância em saúde no HU.
A pesquisadora realizou adendo no protocolo (embora sem documento de encaminhamento) que
corresponde ao aumento no número de participantes na pesquisa. O aumento seria de 300 participantes mudança no n de 200 para 500 participantes).

Considerações sobre os Termos de apresentação obrigatória:

Descreve suficientemente o procedimento para obtenção do TCLE, além de versão reformulada do TLE (TCLE 12.11.2014).

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

Os pesquisadores descreveram detalhadamente o procedimento para obtenção dos TCLEs de forma a documentar, caso a caso, a impossibilidade da sua obtenção. No tocante a esse ponto, o

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FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

Continuação do Parecer: 877.292

protocolo está conforme as exigências pregadas pela Res CNS 466/2012 para a dispensa do TCLE.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

DOURADOS, 19 de Novembro de 2014

Assinado por:
Paulo Roberto dos Santos Ferreira
(Coordenador)

Endereço: Rua Melvin Jones, 940
Bairro: Jardim América **CEP:** 79.803-010
UF: MS **Município:** DOURADOS
Telefone: (67)3410-2853 **E-mail:** cep@ufgd.edu.br

Anexo B. Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

O (a) Senhor (a) está sendo convidado (a) a participar, como voluntário, de uma pesquisa com nome de "Caracterização e epidemiologia molecular de bactérias gram-negativas produtoras de beta-lactamases isoladas de pacientes internados em hospitais de Dourados/MS". Esta pesquisa tem como objetivo estudar as bactérias resistentes a vários antibióticos isoladas de pacientes internados em hospitais de Dourados/MS. Após ler com atenção este documento e ser esclarecido (a) sobre as informações a seguir, e portanto, no caso aceite participar do estudo, assine ao final deste documento, que está em duas vias. Uma delas é sua e a outra é do pesquisador responsável. Em caso de recusa você não será penalizado (a) de forma alguma.

INFORMAÇÕES IMPORTANTES QUE VOCÊ PRECISA SABER SOBRE A PESQUISA:

- Título da pesquisa: "Caracterização e epidemiologia molecular de bactérias gram-negativas produtoras de beta-lactamases isoladas de pacientes internados em hospitais de Dourados/MS".
- Justifica-se a realização desta pesquisa pela importância do estudo de bactérias resistentes a antibióticos para o restabelecimento da saúde do paciente bem como para o controle da disseminação destas bactérias no hospital, buscando evitar novos casos de infecção.
- Objetivos da pesquisa: Identificar bactérias resistentes a diversos antibióticos isoladas de pacientes internados em hospitais de Dourados/MS; estudar a resistência destas bactérias a vários antibióticos; identificar a presença de genes de resistência; identificar os fatores de risco relacionados às infecções causadas pelas bactérias resistentes.
- A pesquisa se baseia na identificação do perfil de resistência das bactérias à vários antibióticos, identificação do gene responsável por esta resistência, estudo do perfil genético e análise dos fatores de risco envolvidos na aquisição de infecções por essas bactérias resistentes.
- A participação do paciente consiste em ler o termo de consentimento, caso concorde participar do estudo, assine o termo, liberando o pesquisador a revisar os dados do seu prontuário. O pesquisador que irá aplicar o TCLE será um aluno de pós-graduação envolvido neste projeto o qual irá explicar ao paciente do que se trata a pesquisa com linguagem acessível ao mesmo.
- Os dados dos prontuários dos pacientes serão utilizados unicamente para esta pesquisa, sendo que a pesquisadora se compromete a manter sigilo absoluto dos dados pessoais dos participantes, sendo que os mesmos serão recodificados de maneira a minimizar os riscos de exposição dos participantes.
- As bactérias serão isoladas de amostras clinicas de pacientes internados em hospitais de Dourados/MS. As amostras clínicas são coletadas apenas e estritamente pelo corpo médico de cada hospital e encaminhadas ao laboratório de microbiologia dos hospitais, onde serão isoladas

as bactérias. ESTE PROCEDIMENTO DE COLETA DE AMOSTRA CLINICA E ISOLAMENTO BACTERIANO FAZ PARTE DA ROTINA DE CADA HOSPITAL e independe da pesquisa proposta aqui.

- As bactérias isoladas nos laboratórios dos hospitais serão encaminhadas ao Laboratório de Pesquisa em Ciências da Saúde da UFGD. **Portanto, o pesquisador não estará envolvido na coleta das amostras dos pacientes, apenas com a revisão dos prontuários e aplicação do TCLE.**
- **O pesquisador garante total sigilo e anonimato que assegure a privacidade dos participantes quanto aos dados coletados dos prontuários.**
- As despesas com a pesquisa são de responsabilidade do pesquisador.
- Não haverá nenhum tipo de pagamento ou gratificação financeira pela sua participação na pesquisa.
- O período de realização da pesquisa será de dezembro de 2013 a maio de 2016.
- É garantida ao sujeito a liberdade de não aceitação, bem como de retirar o consentimento, sem qualquer prejuízo ou dano moral ou continuidade do acompanhamento médico.
- Os resultados da pesquisa irão contribuir para o conhecimento de casos de infecções causadas por bactérias resistentes a vários antibióticos, ajudando no controle de bactérias resistentes nos hospitais envolvidos no estudo e melhoria na qualidade da assistência aos pacientes internados nas instituições hospitalares.
- Em caso de dúvida o (a) Senhor (a) poderá procurar o Comitê de Ética em Pesquisa da Universidade Federal da Grande Dourados localizado na Rua Melvin Jones, 940 - Jardim América, Dourados-MS ou pelo telefone (67) 3410-2053, ou ainda procurar o pesquisador responsável, Dra. Simone Simionatto, que se encontra na Universidade Federal da Grande Dourados (UFGD) - Rodovia Dourados - Itahum, Km 12 - Cidade Universitária ou pelo telefone 3410-2225.

_____

Nome do pesquisador responsável

_____

Assinatura do pesquisador responsável

CONSENTIMENTO PARA A PARTICIPAÇÃO DA PESQUISA

Eu, _____, portador (a) do RG/CPF _____, residente no endereço _____, nº_____, CEP _____, Bairro _____, concordo em participar como voluntário do estudo "Caracterização e epidemiologia molecular de bactérias gram-negativas produtoras de beta-lactamases isoladas de pacientes internados em hospitais de Dourados/MS", sob a responsabilidade da Profa. Dra. Simone Simionatto. Declaro que fui devidamente informado (a) e esclarecido pelo pesquisador _____, sobre a pesquisa, os procedimentos envolvidos, e a importância da minha participação. Foi me garantido que posso desistir da pesquisa a qualquer momento, sem qualquer penalidade ou interrupção do meu acompanhamento/ assistência/ tratamento médico. Lembrando que este documento está em duas vias, uma ficará com o Senhor (a) e outra com o pesquisador responsável.

Local e data: _____

_____

Nome do sujeito ou responsável

_____

Assinatura do sujeito ou responsável

_____

Dra. Simone Simionatto

Universidade Federal da Grande Dourados, Faculdade de Ciências da Saúde.

Rodovia Dourados Itahum Km 12, 79804-970 - Dourados, MS - Brasil - Caixa-Postal: 322.

Telefone: (67) 3410-2225

Declaro que entendi os objetivos, riscos e benefícios de minha participação na pesquisa e concordo em participar.

_____

Nome Completo

Anexo C. Questionário para a avaliação dos prontuários das Unidades de Terapia Intensiva Neonatal e Adulto

Confidential

Acinetobacter Banco

Número do Questionário _____

Record ID _____

Banco:
☐ Neonatal
☐ Adulto

---

1) Identificação do paciente:

RN de (nome da mãe): _____

Nome: _____

Iniciais do Paciente: _____

Sexo:
☐ 1 - Feminino
☐ 2 - Masculino

Data de nascimento: _____

Idade (dias): _____

Idade (anos): _____

Peso ao nascer: _____
(em gramas)

Raça/cor:
☐ 1 - Branco
☐ 2 - Negro
☐ 3 - Pardo
☐ 4 - Indígena

Paciente
☐ 1 - Caso
☐ 2 - Controle

Análise genética:
☐ blaKPC-2
☐ blaIMP-1
☐ blaVIM-1
☐ blaNDM-1
☐ blaOXA-23-like
☐ blaOXA-24-like
☐ blaOXA-51-like
☐ blaOXA-58-like

---

2) Dados hospitalares e variáveis epidemiológicas:

Número do prontuário: _____

Hospital: _____

Convênio: _____

Data da internação: _____

Data da alta: _____

Local antes da admissão:
☐ 1 - Residência
☐ 2 - Instituição de saúde

Motivo da internação:

- ☐ 1 - Prematuridade (IG< 37 semanas)
- ☐ 2 - Gemelaridade
- ☐ 3 - Baixo peso ao nascer (P< 2500g)
- ☐ 4 - Muito baixo peso ao nascer (P< 1500g)
- ☐ 5 - Extremo baixo peso ao nascer (P< 1000g)
- ☐ 6 - Hipoglicemia
- ☐ 7 - Anóxia neonatal
- ☐ 8 - Síndrome da Membrana Hialina (SMH)
- ☐ 9 - Síndrome da Aspiração Meconial (SAM)
- ☐ 10 - Cardiopatia congênita
- ☐ 11 - Outras
- ☐ 12 - Broncodisplasia Pulmonar (BDP)
- ☐ 13 - Pneumonia Neonatal
- ☐ 14 - Infecção Neonatal
- ☐ 15 - Sepse Neonatal
- ☐ 16 - Apneia
- ☐ 17 - Icterícia
- ☐ 18 - Gastrosquise
- ☐ 19 - Mal formação genética

Motivo da internação: _____

Cardiopatia Congênita. Qual? _____

Outras. Qual? _____

Procedência:

- ☐ 1 - Centro Obstétrico HU
- ☐ 2 - Alojamento Conjunto HU
- ☐ 3 - UCI HU
- ☐ 4 - Instituição externa ao HU

Caso tenha vindo de outra instituição de saúde:

Nome da instituição: _____

Tempo de internação: _____ (em dias.)

Data da saída: _____

Houve transferência do paciente entre as unidades hospitalares?

1) Unidade de Origem _____

1) Tempo (unidade de origem) _____ (em dias)

1) Unidade Destino _____

1) Tempo (unidade destino) _____ (em dias)

2) Unidade de Origem _____

2) Tempo (unidade de origem) _____ (em dias)

2) Unidade Destino _____

2) Tempo (unidade destino) _____ (em dias)

Confidential

Tempo de internação:
- ☐ 1 - até 30 dias
- ☐ 2 - 30 a 60 dias
- ☐ 3 - 60 a 90 dias
- ☐ 4 - 90 a 180 dias
- ☐ 5 - mais de 180 dias

Evolução do caso:
- ☐ 1 - Alta hospitalar
- ☐ 2 - Transferência hospitalar
- ☐ 3 - Óbito
- ☐ 4 - Óbito por outras causas

Fatores de risco maternos:
- ☐ 1 - DHEG (Doença Hipertensiva Específica da Gestação
- ☐ 2 - HAS
- ☐ 3 - ICC
- ☐ 4 - DMG (Diabetes Mellitus Gestacional)
- ☐ 5 - DM
- ☐ 6 - ITU (Infecção do Trato Urinário)
- ☐ 7 - DPP (Descolamento Prematuro de Placenta)
- ☐ 8 - Oligodrâmnio
- ☐ 9 - Polidrâmnio
- ☐ 10 - Bolsa Rota (rotura prematura de membranas)
- ☐ 11 - Sífilis
- ☐ 12 - Rubéola
- ☐ 13 - Toxoplasmose
- ☐ 14 - CMV
- ☐ 15 - HIV
- ☐ 16 - Hepatite
- ☐ 17 - Herpes

Co-morbidades:
- ☐ 1 - Diabetes
- ☐ 2 - Alcoolismo
- ☐ 3 - HAS
- ☐ 4 - Úlcera de decúbito
- ☐ 5 - Doença pulmonar obstrutiva crônica
- ☐ 6 - Insuficiência cardíaca
- ☐ 7 - Insuficiência renal
- ☐ 8 - Dependente químico
- ☐ 9 - Insuficiência respiratória
- ☐ 10 - Câncer
- ☐ 11 - Tabagismo
- ☐ 12 - Comprometimento neurológico
- ☐ 13 - Doença neurometabólica
- ☐ 14 - Neoplasias
- ☐ 15 - Doenças hematológicas
- ☐ 16 - Transplante de órgãos
- ☐ 17 - Edema pulmonar
- ☐ 18 - Infecção por HIV
- ☐ 19 - Sepse

Fatores de risco:
- ☐ 1 - Ventilação mecânica
- ☐ 2 - Procedimento cirúrgico
- ☐ 3 - CVC (cateter venoso central)
- ☐ 4 - Cateter urinário
- ☐ 5 - Uso de imunossupressores
- ☐ 6 - Hemodiálise
- ☐ 7 - Uso de antibióticos
- ☐ 8 - Sonda nasogástrica
- ☐ 9 - Drenagem torácica
- ☐ 10 - Diálise peritoneal
- ☐ 11 - Drenagem abdominal

Idade materna: _____

Idade Gestacional (em semanas): _____

Realizou Pré- Natal?
- ☐ 1 - Não
- ☐ 2 - Sim

Confidential

Quantas consultas? _____

Tipo de parto:
☐ 1 - Cesáreo
☐ 2 - Normal

Classificação do neonato quanto ao peso:
☐ 1 - AIG (adequado para idade gestacional)
☐ 2 - PIG (pequeno para idade gestacional)
☐ 3 - GIG (grande para idade gestacional)

Fatores de risco extrínsecos:
☐ 1 - TOT
☐ 2 - CPAP nasal
☐ 3 - AVP
☐ 4 - AVC
☐ 5 - Cateter umbilical
☐ 6 - Sonda vesical
☐ 7 - SNG
☐ 8 - SNE
☐ 9 - DVP
☐ 10 - Outros?

Tempo (data início e término) - TOT _____ (em dias)

Tempo (data início e término) - CPAP nasal _____ (em dias)

Tempo (data início e término) - AVP _____ (em dias)

Tempo (data início e término) - AVC _____ (em dias)

Tempo (data início e término) - Cateter umbilical _____ (em dias)

Tempo (data início e término) - Sonda vesical _____ (em dias)

Tempo (data início e término) - SNG _____ (em dias)

Tempo (data início e término) - SNE _____ (em dias)

Tempo (data início e término) - DVP _____ (em dias)

Outro? Quais? _____

Tempo (data início e término) - Outro? _____

Tipo de Leito:
☐ 1 - Incubadora
☐ 2 - Berço aquecido
☐ 3 - Berço simples

Procedimento cirúrgico:
☐ 1 - Não
☐ 2 - Sim

Em caso de procedimento, relatar qual o tipo de procedimento: _____

Local da cirurgia _____

Confidential

Fonte de Infecção:

- ☐ 1 - Secreção traqueal
- ☐ 2 - Sangue
- ☐ 3 - Ferida
- ☐ 4 - Ponta de Cateter
- ☐ 5 - Swab nasal
- ☐ 6 - Swab retal
- ☐ 7 - Urina
- ☐ 8 - Fezes
- ☐ 9 - Outros

Quais? _____

Colonização por Acinetobacter spp.:

- ☐ 1 - Sim
- ☐ 2 - Não

Qual micro-organismo (espécie Acinetobacter)?

- ☐ A. baumannii
- ☐ A. calcoaceticus
- ☐ A. lwoffii
- ☐ A. johnsonii
- ☐ A. haemolyticus
- ☐ A. junii

Data de Isolamento _____

Exposição prévia a antimicrobianos:

Exposição

- ☐ Yes
- ☐ No

Medicamento:

- ☐ Aminoglicosídeos
- ☐ B-lactamicos/b-lactamase inibidores
- ☐ Carbapenêmicos
- ☐ Cefalosporinas
- ☐ Polimixinas
- ☐ Quinolonas

Anexo D. Short paper aceito na Revista Brasileira de Medicina Tropical

Identification of SPM-1-producing *Pseudomonas aeruginosa* in the Central-West region of Brazil: a case study

Running title: Maciel WG - SPM-producing *Pseudomonas aeruginosa*: a case study.

***Wirlaine Glauce Maciel*[1], *Kesia Esther da Silva*[1], *José Victor Bortolotto Bampi*[1], *Graciela Mendonça dos Santos Bet*[1],[2], *Ana Carolina Ramos*[3], *Ana Cristina Gales*[3] *and Simone Simionatto*[1]**

[1]. ***Laboratório de Pesquisa em Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brasil.*** **[2].** ***Hospital Universitário de Dourados, Universidade Federal da Grande Dourados, Dourados, MS, Brasil.*** **[3].** ***Laboratório ALERTA, Disciplina de Infectologia, Departamento de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brasil.***

Corresponding author: Simone Simionatto. Laboratório de Pesquisa em Ciências da Saúde/Universidade Federal da Grande Dourados, Rodovia Dourados - Itahum, km 12, Cidade Universitária, CEP: 79804970, Dourados, Mato Grosso do Sul, Brazil.

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Abstract

Metallo-beta-lactamase production is an important mechanism for carbapenem resistance of *Pseudomonas aeruginosa*, which represents an emerging public health challenge. We report the case of a patient admitted to an intensive care unit, with sepsis caused by multidrug-resistant São Paulo Metallo-beta-lactamase-1-producing *P. aeruginosa*. This is the first case of infection by this pathogenic strain in the State of Mato Grosso do Sul, Brazil. Thus, infection control measures are required for preventing future spread and outbreaks.

Keywords: *Pseudomonas aeruginosa.* Metallo-beta-lactamase. SPM.

INTRODUCTION

Pseudomonas aeruginosa is a leading cause of nosocomial infections, particularly in intensive care units (ICUs). The prevalence of carbapenem resistance in these bacteria has been increasing worldwide, especially in Brazil[1]. The high frequency of genetic mutations that confer antibiotic resistance to these pathogens is a major concern in hospitals worldwide. The detection of multiple antimicrobial resistance determinants in these microorganisms is increasing. The production of metallo beta-lactamase (MβL) by *P. aeruginosa* strains is considered one of the most important factors conferring resistance to β-lactam antibiotics, including carbapenems[2].

Several types of MβL have been identified among *P. aeruginosa* strains. Production of São Paulo metallo-betalactamase (SPM) has been associated with broad-spectrum β-lactam resistance, including carbapenem resistance, and has been identified as the predominant MβL in Brazil[1] [3]. SPM-1 was first detected and reported in 2001 in São Paulo, Brazil[1]. Since then, SPM-1-producing *P. aeruginosa* strains have been reported in different regions of Brazil[3] [4]. However, data are lacking regarding the clinical features of SPM-1-related drug resistance in the Central Western Brazilian State of Mato Grosso do Sul. Here, we report a multidrug-resistant *P. aeruginosa* strain producing SPM-1, which was isolated from a patient hospitalized in the ICU of a teaching hospital in the City of Dourados, Mato Grosso do Sul. The rapid identification of multidrug resistance is essential to prevent the spread of these microorganisms in hospitals, and to establish adequate therapies against this infection[5].

CASE REPORT

In November 2014, a 57-year-old man was admitted to the ICU of a teaching hospital in the City of Dourados, Mato Grosso do Sul, a Central Western Brazilian state. The patient had been hospitalized for 6 days in another health facility where, owing to Fournier gangrene,

he underwent a colostomy procedure and surgical debridement. The patient was admitted to our institution because of hemiplegia on the left side, which was caused by a previous stroke. He also displayed a low level of consciousness (Glasgow coma score of 6) and had a history of hypertension and diabetes. He was sedated and placed on mechanical ventilation via tracheostomy. On the day of admission, he underwent dialysis because of acute renal failure with suspicion of integument and urinary sepsis, which presented as lower limb edema and the discreet presence of debridement in the expansion region of the scrotum. He underwent procedures to introduce several invasive devices such as a central venous catheters, colostomy bag and urethral.

After 34 days of hospitalization, *P. aeruginosa* was isolated from urine culture samples. Intravenous treatment, which included teicoplanin (600 mg) once a day and meropenem (1.000 mg) twice a day, was administered for 26 days. On the 21st and 39th days of hospitalization, the patient experienced cardiopulmonary arrest without electrical activity or pulse; however, the medical staff managed to revive him. After 49 days of hospitalization, his condition had progressively worsened and *P. aeruginosa* was isolated from another urine sample. New antibiotic therapy was started with meropenem (500 mg) and tigecycline (50 mg) twice a day for 19 days. On the 60th day, the patient collapsed and experienced two heart attacks that progressed to death. The cause of death was reported as cardiorespiratory arrest aggravated by sepsis.

The *P. aeruginosa* strains were identified using the automated microbial testing system VITEK® 2 (bioMérieux, Marcy-l′Étoile, France) and confirmed by performing matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT spectrometer (Bruker Daltonics, Billerica, MA, USA)[6]. The minimal inhibitory concentrations (MICs) were determined using broth microdilution according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[7], except for tigecycline MICs,

which were interpreted using the Food and Drug Administration guidelines[8]. *P. aeruginosa* showed sensitivity only to polymyxin B (MIC_{50} 0.5 μg/mL), colistin (MIC_{50} 4 μg/mL) and tigecycline (MIC_{50} 0.5 μg/mL). Two *P. aeruginosa* strains isolated had the same antimicrobial resistance profile.

Preliminary screening for the presence of carbapenemases was performed using the modified Hodge test according to CLSI guidelines[7] and by ertapenem hydrolysis using MALDI-TOF MS[9]. The presence of genes encoding β-lactamase (*bla*$_{IMP-1}$, *bla*$_{KPC-2}$, *bla*$_{NDM-1}$, *bla*$_{VIM-1}$, *bla*$_{OXA-48}$, *bla*$_{GES-1}$ and *bla*$_{SPM-1}$) was detected using polymerase chain reaction (PCR), followed by sequencing using specific primers as previously described[6]. The *bla*$_{SPM-1}$ gene was present in the multidrug-resistant *P. aeruginosa* strains. The predicted protein sequence was analyzed using the Lasergene Software Package (DNASTAR, Madison, WI, USA) and compared to the sequences deposited in GenBank. The presence of *bla*$_{IMP-1}$, *bla*$_{NDM-l}$, *bla*$_{VIM-1}$, *bla*$_{OXA-48}$ and *bla*$_{GES-1}$ could not be confirmed.

Ethicals considerastions

This study was conducted with the approval of the Research Ethics Committee from the Universidade Federal da Grande Dourados (no. 877.292/2014) and in accordance with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000. The patient involved in the study provided written informed consent prior to participation.

DISCUSSION

The spread of carbapenemase-producing *P. aeruginosa* strains is alarming, because this species is a main source of hospital-acquired infections in critically ill patients, and is known for its ability to transfer drug resistance genes[2]. The dissemination of carbapenemase-producing strains is of great concern to public health services in Brazil. Several outbreaks and

sporadic cases of SPM-1-producing strains, which spread rapidly, have been reported in Brazil[10][11]. However, to our knowledge, there have been no reports of SPM-1-producing *P. aeruginosa* strains in the State of Mato Grosso do Sul. Here, we report a case of a patient who died from uncontrolled infection complicated by sepsis and a urinary tract infection, which was originally caused by a multidrug-resistant SPM-1- producing *P. aeruginosa* strain. The patient had not traveled to any other state in which these pathogens have been previously reported. However, he was exposed to risk factors associated with acquisition of MβL-producing bacteria, such as an ICU stay, extended hospitalization, history of comorbidity and the use of invasive devices[9] [11]. This patient was not colonized at admission. His clinical history showed previous hospitalizations and surgical procedures before our initial culture was collected. However, this study was not able to identify the introduction of this strain into our hospital. Phenotypic test results were concordant with the detection of carbapenemase, and PCR and deoxyribonucleic acid (DNA) sequencing confirmed SPM-1- producing *P. aeruginosa*.

The patient died after administration of two different antimicrobial therapies. The initial treatment consisted of a combination of teicoplanin and meropenem. However, this inappropriate therapy may have contributed to the worsening of his clinical condition and the evolution of sepsis. In vitro test results suggested that polymyxin B and colistin represent alternative therapies. Although these drugs may be associated with severe nephrotoxicity and there is a paucity of data on clinical outcomes, they are still used as the last therapeutic choice against *P. aeruginosa* infections[12].

In this study, we confirmed the presence of SPM-1- producing *P. aeruginosa* in Mato Grosso do Sul, a Central Western Brazilian state. This report highlights the potential emergence of these strains, and demonstrates the need for strategies to improve infection control measures that prevent an increase in these nosocomial infections. The rapid

identification of resistance profiles of carbapenemase-producing strains is crucial for timely implementation of appropriate antimicrobial therapy.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Financial support

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Anexo E. Normas da Revista Brasileira de Medicina Tropical

Revista da Sociedade Brasileira de Medicina Tropical
Journal of the Brazilian Society of Tropical Medicine

ISSN 0037-8682 ***printed version***
ISSN 1678-9849 ***online version***

INSTRUCTIONS TO AUTHORS

Scope

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Review policy

Manuscripts submitted for publication in the **Revista da Sociedade Brasileira de Medicina Tropical**are initially evaluated by the administrative staff to ensure compliance with journal guidelines. Those manuscripts that meet the basic requirements will then be sent to at least two reviewers for evaluation through a peer review system. The journal editors will use the reviewers' detailed reports to decide whether to accept a submitted paper. If there is a difference of opinion amongst the reviewers, the manuscript will be sent to a third reviewer to validate a final editorial decision, in accordance with the submission workflow of the **Revista da Sociedade Brasileira de Medicina Tropical** (available online at http://www.scielo.br/revistas/rsbmt/iinstruc.htm#005).

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Manuscript types

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Major Articles: Major article manuscripts must report original

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Review Articles: Review articles should present a critical review of recent trends and progress in the study of infectious diseases, rather than a mere literature review; such articles are usually written at the direct request of the editor. Review articles have a 3,500-word limit (excluding the abstract, title and references) and should be accompanied by a non-structured abstract of up to 250 words; Five illustrations (tables and figures) are allowed. The **Revista da Sociedade Brasileira de Medicina Tropical** also publishes mini-reviews. These articles have a 3,000-word limit (excluding the abstract, title and references) and must be accompanied by a non-structured abstract of up to 200 words; mini-reviews may contain up to three illustrations (tables and figures). The manuscript should be organized using the following headings: Title, Running Title, Abstract (non-structured), Manuscript Text, Conflict of Interest Statement, References List and Figures Titles/Legends.

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Case Reports: Case reports must be brief reports of up to 1,500 words, with a maximum of three illustrations (tables and figures), up to 12 references, and a non-structured abstract of up to 100 words. The body of the manuscript should be divided according to the following headings: Introduction, Case Report, Discussion, References and Figures title/legend.

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Images in Infectious Diseases: An "Images in Infectious Diseases" submission must include up to three photographs of the best quality possible. Only three authors and three references are allowed. The maximum length of such an article is 250 words (excluding title and references), with an emphasis on figure description. The themes should involve some clinical lesson and provide appropriate illustrations and figure descriptions to clarify that lesson.

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Authors are advised to read these instructions carefully and follow them closely to ensure the timely and efficient review and publication of their manuscript. The Editors reserve the right to return manuscripts that do not adhere to these guidelines.

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Authors' contribution: The authors must include, in a separate document, a statement of authorship responsibility, specifying the contribution of each author to the study.

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Manuscript layout

The manuscript should be prepared using standard word processing software and should use Times New Roman size 12 font with double spacing throughout the text, figure title/legends, and references, with margins of at least 3 cm. Divide the manuscript into the following sections: Cover Letter (addressed to the Editor-in Chief), Title Page, Manuscript Title, Running title, Abstract, Keywords, Manuscript Text, Conflict of Interest statement, Acknowledgments, Financial Support,

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Title Page: The title page should include the authors' names in direct order and without abbreviations, along with institutional affiliations (e.g. each author's department, institution, city, state, and country). The complete address of the corresponding author must be specified, including telephone and fax numbers and e-mail address. Financial Support and Acknowledgments could be included in Title page too. The number of authors and co-authors per paper should be limited to eight, except for multicenter studies.

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Structured Abstract: The structured abstract should summarize the results obtained and the major conclusions in such a way that a reader not already familiar with the particular topic of the paper can understand the implications of the article. The abstract should not exceed 250 words (100 words in the case of short communications) and must be structured according to the following headings: *Introduction, Methods, Results,* and *Conclusions.* Abbreviations should be avoided.

Keywords: Three to five important keywords should be listed in English immediately below the structured abstract.

Introduction: The article's introduction should be brief, setting out the purposes for which the study has been performed. Any relevant previous studies should be cited only when essential.

Methods: The methods used in the study should be reported in sufficient detail that readers and reviewers can understand precisely what has been done; any description should be thorough enough to allow repetition or replication by others. Standard techniques only need to be referenced; detailed descriptions are not required.

Ethics: When reporting on experiments on human beings, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional, regional, or national) and in keeping with the Helsinki Declaration of 1964, as revised in 1975, 1983, 1989, 1996, and 2000. When reporting on experiments on animals, indicate whether a national research council's guide for animal experimentation or any law on the care and use of laboratory animals was complied with and the approval number must be sent to the journal.

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Acknowledgments: Any acknowledgments should be short, concise, and restricted to those that are absolutely necessary. In case of sponsoring institutions, do not use acronyms.

Conflict of Interest: All authors should disclose any type of conflict of interest during the development of the study.

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Russell FD, Coppell AL, Davenport AP. *In vitro* enzymatic processing of radiolabelled big ET-1 in human kidney as a food ingredient. Biochem Pharmacol 1998; 55:697-701.

2. Book chapter: Authors' book chapter, Chapter title, Editors, Book name, Edititon, City, Publisher, Year and chapter pages.
Porter RJ, Meldrum BS. Antiepileptic drugs. *In:* Katzung BG, editor. Basic and clinical pharmacology. 6th ed. Norwalk (CN): Appeleton and Lange; 1995. p. 361-380.

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Anexo F. Normas da Revista *Journal of Hospital Infection*

Introduction

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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Commentaries

http://www.journalofhospitalinfection.com/content/authorinfo

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.

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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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We welcome letters relating to, or responding to, recently published items in the journal. Where appropriate, these will be shown to the authors of the original article, who will be invited to respond. We also welcome correspondence relating to general observations about infection prevention and control practice. We will only publish reports of research as a letter where the findings are important or radical enough to merit the earliest possible publication.

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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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Authorship

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This journal has an embargo period of 12 months.

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All newly submitted papers are first considered by the Editorial team.

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