Pontifícia Universidade Católica do Rio Grande do Sul

Faculdade de Biociências

Programa de Pós-Graduação em Biologia Celular e Molecular

Otávio Hallal Ferreira Raro

Identificação e determinação de resistência antimicrobiana em isolados nosocomiais de *Acinetobacter baumannii*

Porto Alegre

Setembro, 2011

Otávio Hallal Ferreira Raro

Identificação e determinação de resistência antimicrobiana em isolados nosocomiais de *Acinetobacter baumannii*

Dissertação de Mestrado apresentado ao Programa de Pós-Graduação em Biologia Celular e Molecular, da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Orientadora: Profa. Dra. Sílvia Dias de Oliveira

Co-orientador: Prof. Dr. Carlos Alexandre Sanchez Ferreira

Porto Alegre

2011

OTÁVIO HALLAL FERREIRA RARO

Identificação e determinação de resistência antimicrobiana em isolados nosocomiais de *Acinetobacter baumannii*

Dissertação de Mestrado apresentado ao Programa de Pós-Graduação em Biologia Celular e Molecular, da Faculdade de Biociências da Pontifícia Universidade Católica do Rio Grande do Sul.

Aprovado em_____de_____

de_____

BANCA EXAMINADORA:

Gertrudes Corção

Marjo Cadó Bessa

Maurício Reis Bogo

Porto Alegre

2011

Agradecimentos

À Profa. Dra. Sílvia Dias de Oliveira agradeço pela oportunidade de ter trabalhado no seu laboratório e por ter dividido comigo valiosos conhecimentos em nossas conversas sobre os mais diversos assuntos durante toda minha carreira acadêmica. Também agradeço pela confiança em mim e no meu trabalho e pela dedicação e cuidado com que conduziu a minha orientação durante estes dois anos.

Aos Professores Dr. Carlos Alexandre Sanchez Ferreira e Dra. Renata Medina pelo apoio em momentos importantes para que este trabalho terminasse com sucesso, assim como pelos conhecimentos compartilhados que contribuíram para o aumento da qualidade científica desta pesquisa.

Aos Professores Dr. João Riboldi e Dra. Christiane Heemann pelo conhecimento compartilhado e pelo serviço prestado para que este trabalho pudesse ser concluído com sucesso.

Ao Serviço de Controle de Infecção do Hospital São Lucas da PUCRS, principalmente à médica Ana Maria Sandri e à enfermeira Luciana Alcântara, e ao Departamento de Microbiologia do Laboratório de Patologia Clínica deste mesmo Hospital, por todo apoio prestado durante o período deste curso.

Aos meus pais, José Carlos e Márcia, não tenho palavras suficientes para agradecer a compreensão e o apoio incondicional durante toda a minha vida acadêmica e por nunca terem medido esforços para me ajudar nesta e em outras conquistas. Sem vocês certamente eu não teria conseguido chegar até aqui.

Às minhas irmãs, Luiza, Thaís e Júlia pelo carinho, pelo companheirismo e pelo apoio durante estes anos de estudo, os quais tornaram esta etapa mais tranquila. Assim como meu avô José Carlos Porres Ferreira Raro e meus tios Eduardo, Rogério e Ronaldo, que sempre foram presentes em quaisquer adversidades.

Aos meus colegas do Laboratório de Imunologia e Microbiologia, especialmente à mestranda, colega e amiga Stephanie Wagner Gallo pelo companheirismo, amizade e compartilhamento de ideias durante todo o meu período acadêmico e a todas as pessoas

que de alguma forma, direta ou indiretamente, contribuíram para que este trabalho tivesse êxito.

Resumo

Acinetobacter baumannii é um importante patógeno oportunista comumente associado a infecções nosocomiais, especialmente em pacientes hospitalizados em unidades de tratamento intensivo (UTIs). Este organismo é reconhecido por sua capacidade de sobreviver em condições adversas no ambiente por períodos prolongados, bem como de facilmente adquirir resistência a drogas antimicrobianas. Atualmente, a crescente resistência antimicrobiana de *A. baumannii* tem constituído um grande desafio para a comunidade médica, uma vez que existem poucas opções efetivas para o tratamento de infecções causadas por este microrganismo. O objetivo deste estudo foi avaliar a presença de *A. baumannii* no ambiente de uma UTI e caracterizar a resistência a drogas antimicrobianas dos isolados obtidos, bem como de isolados de pacientes internados na UTI do mesmo hospital no qual as amostras ambientais foram coletadas. Para tanto, 886 amostras ambientais e de luvas foram coletadas de uma UTI do Hospital São Lucas, Porto Alegre, Brasil, e 46 isolados clínicos foram obtidos no Laboratório do mesmo hospital. Após a identificação dos isolados como *A. baumannii* através de PCR utilizando como alvos os genes rDNA 16S e $bla_{\text{OXA-51}}$, foram determinadas a resistência a 20 drogas antimicrobianas previstas pelo CLSI e a produção de metalo-beta-lactamases em isolados com suscetibilidade reduzida aos carbapenêmicos. Também foi avaliada a presença de integrons e dos genes $bla_{\text{OXA-23}}$ e bla_{IMP} através de PCR. *A. baumannii* foi identificado em 9,6% das amostras ambientais e de luvas coletadas. Obteve-se um alto percentual de isolados multirresistentes (MDR), assim como foram detectadas alta taxas de suscetibilidade reduzida aos carbapenêmicos. Todos os 89 isolados que apresentaram integrons foram MDR. Dentre os isolados com suscetibilidade reduzida aos carbapenêmicos, todos apresentaram o gene $bla_{\text{OXA-23}}$, e 41,4% não-clínicos e 54% clínicos carrearam o gene bla_{IMP}. Alta resistência à polimixina B foi detectada, principalmente em isolados não-clínicos. Embora alta prevalência de resistência antimicrobiana tenha sido encontrada em isolados clínicos e não clínicos, os últimos constituem grande preocupação, pois podem indicar o ambiente hospitalar como um reservatório de *A. baumannii* MDR.

Palavras-chave: *Acinetobacter baumannii*; Resistência antimicrobiana; β-lactamases; Infecções hospitalares; Carbapenêmicos

Abstract

Acinetobacter baumannii is an important opportunistic pathogen commonly associated with nosocomial infections, especially in patients hospitalized in intensive care units (ICUs). This microorganism is renowned for its ability to survive under adverse conditions in the environment for extended periods, as well as to rapidly acquire resistance to antimicrobial drugs. Nowadays, the increasing antimicrobial resistance of *A. baumannii* has been a great challenge for the medical community, since there are few effective options for the treatment of infections caused by this organism. The aim of this study was to evaluate the presence of the *A. baumannii* from an ICU environment and to characterize the antimicrobial drug resistance of the isolates obtained, as well as of the isolates from patients in ICU of the same hospital in which it was collected environmental samples. For this, 886 environmental and gloves samples were collected from an ICU of São Lucas Hospital, Porto Alegre, Brazil, and 46 clinical isolates were obtained from the Laboratory of the same hospital. After the identification of the isolates as *A. baumannii* by PCR using as target 16S rDNA and $bla_{\text{OXA-51}}$ genes, the resistance to 20 antimicrobial drugs and the production of metallo-beta-lactamases were evaluated in isolates presenting carbapenem reduced susceptibility. Also, it was evaluated the presence of integrons and $bla_{\text{OXA-23}}$ and bla_{IMP} genes by PCR. *A. baumannii* was identified in 9.6% of environmental and glove samples collected. High percentage of multiresistant (MDR) isolates was found, as well as it was detected high rates of reduced susceptibility to carbapenems. All 89 isolates integron positive were MDR. Between isolates with reduced susceptibility to carbapenems, all presented $bla_{\text{OXA-23}}$, and 41.4% non-clinical and 54% clinical carried the bla_{IMP}. High resistance to polymyxin B was detected, mainly in non-clinical isolates. Although high prevalence has been found in clinical and non-clinical isolates, the latter constitute a great concern, because they can indicate the hospital environment as a reservoir of MDR *A. baumannii*.

Keywords: *Acinetobacter baumannii*; Antimicrobial drug resistance; β-lactamases; Hospital infections; Carbapenems

Lista de abreviações

13TU – 13 Tjernberg and Ursing

AIM – Austrália imipenemase

AmpC – beta-lactamase cromossômica

Ari - Acinetobacter resistant to imipenem

AFLP - Amplified fragment length polymorphism

API 20 NE - Analytical profile index for non-enteric Gram-negative rods

***bla* –** beta-lactamase

BHI – Brain heart infusion

BSAC - Sociedade Britânica para a Quimioterapia Antimicrobiana

CHDL – beta-lactamase hidrolizante de carbapenêmicos

CRAB – *Acinetobacter baumannii* resistente aos carbapenêmicos

CTI – Centro de Tratamento Intensivo

DIM - Dutch imipenemase

EDTA - Ácido etilenodiamino tetra-acético

ESBL – beta-lactamase de espectro estendido

ES-OXA – beta-lactamase de amplo espectro

GIM – German imipenemase

***gyrB* –** DNA girase subunidade B

HSL – Hospital São Lucas

IMP - Imipenemase

ISAba - Sequência de Inserção de *Acinetobacter baumannii*

KHM - Kyorin Hospital metalo-beta-lactamase

KPC - *Klebsiella pneumoniae* carbapenemase

MDR – Multidrug Resistance

MYSTIC - *Meropenem Yearly Susceptibility Test Information Collection*

MβL – Metalo-beta-lactamase

NaCl – Cloreto de Sódio

NDM - New Delhi metalo-beta-lactamase

NS-OXA – beta-lactamase de estreito espectro

OXA - Oxacilinase

PBP – Proteína Ligadora de Penicilina

PBP2 – Proteína Ligadora de Penicilina tipo 2

PCR – Reação em cadeia pela Polimerase

PUCRS – Pontifícia Universidade Católica do Rio Grande do Sul

rDNA – DNA ribossômico

SENTRY - SENTRY antimicrobial surveillance programme

SIM - Seoul imipenemase

SPM – São Paulo metalo-beta-lactamase

TSI – Triple Sugar Iron

UNIFESP - Universidade Federal de São Paulo

UTI – Unidade de Tratamento Intensivo

VEB - Vietnamese extended-spectrum beta-lactamase

VIM – Verona imipenemase

XDR - Extreme Drug Resistance

Sumário

Capítulo 1

Introdução

Objetivos

1.1 Introdução

Acinetobacter spp. começaram a ser significativamente reconhecidas como patógenos nosocomiais durante a década de 1970. Nos primeiros estudos *in vitro* relacionados a essas bactérias, a maior parte dos isolados clínicos apresentava um perfil suscetível aos antimicrobianos comumente utilizados, consequentemente, as infecções ocasionadas por esses organismos eram relativamente fáceis de serem tratadas (1). Porém, relatos de isolados clínicos de *Acinetobacter* spp. multirresistentes (MDR) têm sido cada vez mais frequentes durante as duas últimas décadas como consequência do uso indiscriminado de agentes antimicrobianos de amplo espectro em hospitais de todo o mundo (2).

Bactérias do gênero *Acinetobacter* são cocobacilos Gram negativos usualmente dispostos em pares, não-fermentadores, aeróbios estritos, não pigmentados no crescimento em agar sangue, imóveis, catalase positivos, oxidase negativos (3) e produtores de um lipopolissacarídeo com alta toxicidade (4). Aproximadamente 30% das cepas de *Acinetobacter* também produzem um exopolissacarídeo, que é um importante fator de virulência responsável pela proteção da bactéria contra as defesas do hospedeiro, sendo implicado em infecções letais em camundongos e em citotoxicidade para células fagocíticas (4,5).

O gênero *Acinetobacter* é classificado, com base na similaridade de sequências de DNA, em 32 "espécies genômicas", das quais 17 são nomeadas e as demais referidas como números (6,7). *Acinetobacter baumannii, Acinetobacter calcoaceticus* e as genospécies não nomeadas 3 e 13TU (Tjernberg and Ursing) são muito similares geneticamente e de difícil distinção fenotípica, sendo agrupadas no complexo *A. baumannii-A. calcoaceticus* (4,6-9). Este grupo representa as espécies de *Acinetobacter* mais comumente associadas com infecções nosocomiais, representando 75% das *Acinetobacter* spp. isoladas de espécimes clínicos (10), sendo, além disso, descrito como carreador de resistência a múltiplas drogas antimicrobianas (11).

O *A. baumannii* é um patógeno oportunista comumente envolvido em infecções nosocomiais, como bacteremias, pneumonia associada à ventilação mecânica, sepse, infecções de feridas, infecção do trato urinário e meningite pós-neurocirurgia, principalmente em pacientes internados em Centros de Tratamento Intensivo (CTIs) (8,12-19). Este patógeno é responsável por cerca de 80% das infecções relatadas

causadas por *Acinetobacter* spp. (17,18). De acordo com dados divulgados pelo Programa de Vigilância Antimicrobiana SENTRY, dentre as três espécies de *Acinetobacter* isoladas com maior frequência na América Latina, o *A. baumannii* representou 75% (20). O *A. baumannii* tem sido responsável por surtos em Unidades de Tratamento Intensivo (UTIs) brasileiras desde 1996 (21) e tornou-se particularmente importante no Brasil devido à sua prevalência e aos seus padrões de resistência (21). De acordo com o *Meropenem Yearly Susceptibility Test Information Collection* (MYSTIC), o *A. baumannii* foi o segundo patógeno mais prevalente em pacientes hospitalizados de sete UTIs em quatro cidades brasileiras e apresentou elevada taxa de resistência a todos os antimicrobianos testados (22).

O *A. baumannii* tem sido isolado do solo, água, animais e humanos, podendo ser habitante normal da pele de seres humanos na comunidade. Além disso, estes microrganismos são comumente isolados em hospitais, onde se disseminam entre os pacientes e no ambiente, sendo frequentemente isolados do trato respiratório de pacientes hospitalizados (23,24). O *A. baumannii* é resistente a diferentes condições adversas do ambiente e, durante os surtos, tem sido recuperado de vários locais em que os pacientes se encontram, tais como camas, móveis e equipamentos hospitalares (25). O tempo de sobrevivência do *A. baumanii* é maior quando a bactéria está suspensa em sangue ou soro quando comparado com a imersão em água destilada (26), sendo relatado como persistente por mais de 5 meses em objetos hospitalares (17,23,27). Estes microrganismos podem se disseminar pelo ar, em curtas distâncias por gotículas de água e em escamações da pele de pacientes que estão colonizados (28), mas o modo mais comum de transmissão é através das mãos dos trabalhadores dos hospitais (8). A identificação desta bactéria pode ser realizada através de provas bioquímicas básicas, como a detecção de hemólise em agar sangue de carneiro, redução de nitrato a nitrito, utilização de citrato de sódio e outras fontes de carbono, crescimento em caldo BHI à 44°C, coloração de Gram, resistência à penicilina, prova da catalase, atividade da urease, hidrólise da gelatina, fermentação de glicose, lactose e/ou sacarose e teste da oxidase (3,29,30). Uma alternativa para a caracterização bioquímica deste microrganismo é a utilização de sistemas comerciais semi-automatizados, como API 20NE, VITEK 2, Phoenix e MicroScan Walkaway. Técnicas moleculares, como AFLP, análise da sequência da região intergênica 16S-23S rDNA, detecção dos genes *gyrB* e $bla_{\text{OXA-51}}$ através da PCR também têm sido utilizadas para a identificação de *A. baumannii* (13,31-37).

Pacientes imunodeprimidos, apresentando sérias doenças concomitantes, e pacientes sujeitos a procedimentos invasivos e tratados com drogas antibacterianas de amplo espectro têm sido acometidos por cepas de *A. baumannii* MDR (38), tendo sido relatadas em hospitais na Europa, Estados Unidos, China, Hong Kong, Coréia, Japão, Brasil, Argentina, Taiwan, bem como em outras áreas do mundo (39-41). A emergência do *A. baumanii* MDR tem sido atribuída à sua rápida capacidade de adquirir determinantes de resistência, pois é bastante suscetível à transmissão gênica horizontal, pertencendo a uma categoria de bactérias Gram negativas que são naturalmente transformáveis (42,43). Além disso, muitas vezes, este microrganismo carreia integrons (44-48), sendo os integrons de classe 1, 2 e 3 envolvidos na conferência de resistência antimicrobiana (49,50). Embora uma estratégia de vigilância ativa não tenha sido amplamente aplicada para *A. baumannii* MDR, estima-se que esse microrganismo seja responsável por 2 a 10% de todas as infecções bacterianas causadas por Gram negativos em UTIs na Europa e nos Estados Unidos (18). Os dados de um estudo de vigilância da Sociedade Britânica para a Quimioterapia Antimicrobiana (BSAC) ilustraram um aumento na resistência antimicrobiana do *A. baumanii* desde 2002, com mais de 30% dos isolados de bacteremia em 2005 sendo resistentes à gentamicina e à piperacilina/tazobactam (51). Entretanto, este mesmo estudo identificou baixas taxas de resistência ao imipenem, não excedendo a 6%. No programa de vigilância antimicrobiana SENTRY Ásia-Pacífico, dados obtidos entre 2001 e 2004 mostraram 26% de resistência ao imipenem, 48% à ceftazidima, 45% à ciprofloxacina e 41% à ampicilina/sulbactam. No programa SENTRY América Latina, as taxas de resistência para o mesmo período foram 14%, 68%, 65% e 48%, respectivamente (52).

Devido à maior incidência de bacilos Gram negativos resistentes a cefalosporinas de amplo espectro ocorrer em ambiente hospitalar, têm sido utilizados os carbapenêmicos (53), uma vez que esses agentes são importantes opções terapêuticas utilizadas no tratamento de infecções nosocomiais devido à sua elevada afinidade pelas proteínas ligadoras de penicilina do tipo 2 (PBP2), estabilidade a muitas beta-lactamases, incluindo as beta-lactamases de espectro estendido (ESBL) e as cromossômicas (AmpC), e excelente permeabilidade através da membrana externa bacteriana (54). No entanto, recentemente, em um isolado clínico de *A. baumannii* suscetível aos carbapenêmicos e produtor de ESBL, uma grande ilha de resistência antimicrobiana com mais de 40 genes de resistência foi identificada, demonstrando a

plasticidade genética do *A. baumannii*, o que proporciona a esta bactéria a capacidade de se beneficiar de uma variedade de mecanismos de resistência quando a pressão seletiva é constante, como ocorre em um ambiente hospitalar (23).

A utilização excessiva dos carbapenêmicos no tratamento de pacientes hospitalizados gera uma pressão seletiva sobre a microbiota nosocomial, favorecendo a seleção de subpopulações de microrganismos com sensibilidade diminuída ou resistente a essas drogas. Desta forma, o *A. baumannii* tem se tornado cada vez mais resistente aos carbapenêmicos, restando poucas opções de tratamento para infecções causadas por esse microrganismo (41,55). Dados reportados indicam que dentre os isolados de *Acinetobacter* spp. causadores de infecções na América Latina, 15,8 a 17% são resistentes aos carbapenêmicos (52). Em um estudo com isolados brasileiros de microrganismos do complexo *A. baumannii-A. calcoaceticus*, observou-se que 25 a 45% foram resistentes aos carbapenêmicos (19), enquanto outro estudo brasileiro, que avaliou somente *A. baumannii*, reportou que 15% a 25% apresentaram resistência aos carbapenêmicos (CRAB) (41). Em Porto Alegre, o primeiro isolado CRAB foi identificado em 2004, sendo, subsequentemente, reportado em departamentos de saúde locais um surto sem precedentes envolvendo dezesseis hospitais e mais de quinhentos casos entre 2004 e 2008 (56).

A resistência aos carbapenêmicos pode dever-se a diferentes mecanismos: produção de beta-lactamases; diminuição da permeabilidade de membranas externas, provavelmente devido à perda ou modificação das porinas; alteração da afinidade de proteínas ligadoras de penicilina (PBPs) e, raramente, pela hiper-expressão de bombas de efluxo (57-59). No entanto, a principal forma de resistência aos carbapenêmicos é mediada pela expressão de beta-lactamases, tais como metalo-beta-lactamases (MβL) e, principalmente, oxacilinases (60-63).

As beta-lactamases têm sido divididas em quatro classes (A, B, C e D), de acordo com a classificação de Ambler, que se baseia nas sequências de nucleotídeos e de aminoácidos destas enzimas. As beta-lactamases pertencentes às classes A, C e D são serina beta-lactamases, enquanto as pertencentes à classe B são metalo-beta-lactamases, que contêm um ou dois zincos no sítio ativo (64).

Dentre as diferentes beta-lactamases da classe A de Ambler, a enzima VEB-1, codificada pelo gene $bla_{\text{VEB-1}}$, é considerada emergente por sua presença ter sido reportada em muitos organismos Gram negativos de diferentes partes do mundo na

última década (65,66). O *bla*$_{\text{VEB-1}}$ constitui, muitas vezes, parte de um cassete gênico presente em um integron de classe 1 (67,68), que foi detectado em cepas de *A. baumannii* isoladas na França e na Argentina (66,67). A enzima KPC, codificada pelo gene *bla*$_{\text{KPC}}$, foi detectada primeiramente em isolados de *Klebsiella pneumoniae* (69), e, recentemente, foi detectada em isolados clínicos de *Acinetobacter* spp. em Porto Rico (70). Esta observação aumenta a importância clínica desses microrganismos, uma vez que as carbapenemases KPC hidrolisam todas as classes de beta-lactâmicos (71).

As enzimas pertencentes à classe C de Ambler são cefalosporinases codificadas em cromossomos de muitas *Enterobacteriaceae* e em alguns outros organismos (72). Os representantes deste grupo são divididos em: subclasse 1, formada por AmpC, P99, ACT-1, CMY-2, FOX-1 e MIR-1, e subclasse 1e, formada por GC1 e CMY-37 (73). A enzima AmpC é a cefalosporinase mais reportada em *Acinetobacter* spp., tendo sido descritas mais de 25 variedades dessa beta-lactamase com ≥94% de similaridade na sequência de proteínas (72,74)

As MßLs são carbapenemases importantes clinicamente e foram detectadas no mundo todo, mas com maior prevalência no sudeste da Ásia e Europa (75,76). Estas beta-lactamases hidrolisam todos os beta-lactâmicos comercialmente disponíveis, sendo a única exceção o monobactam, aztreonam (77). Essas enzimas são inibidas pelo ácido etilenodiaminotetracético (EDTA) ou por compostos derivados do ácido tiolático, não sendo comumente inibidas por inibidores de serino-beta-lactamases disponíveis comercialmente, como o sulbactam, tazobactam e ácido clavulânico (78).

As MßLs são produzidas intrinsecamente por alguns microrganismos, como *Bacillus cereus* (79), *Chryseobacterium meningosepticum* (80), *Stenotrophomonas maltophilia* (81-83), *Chryseobacterium indologenes*, *Legionella gormanii*, *Caulobacter crescentus* (84) e *Aeromonas* spp. (85-87). Entretanto, desde o início da década de 1990, genes que codificam MβL têm sido descritos em microrganismos clinicamente importantes, como *Acinetobacter* spp., *Pseudomonas* spp. e membros da família *Enterobacteriaceae* (88). Os genes que codificam para as MßLs foram encontrados inseridos em elementos genéticos móveis, fazendo com que essas enzimas passassem a ser conhecidas como MßLs móveis ou adquiridas. Atualmente, são conhecidas nove subclasses de MßL adquiridas: IMP (imipenemase) (89), VIM (Verona imipenemase) (90), SPM (São Paulo metalo-beta-lactamase) (91), GIM (German imipenemase) (92), SIM-1 (Seoul imipenemase) (93), NDM-1 (New Delhi metalo-beta-lactamase) (94), AIM-1 (Australia imipenemase) (95), KHM-1 (Kyorin Hospital metalo-beta-lactamase)

(96) e DIM-1 (Dutch imipenemase) (97). A maioria dos genes que codifica enzimas tipo IMP, VIM, GIM, NDM, DIM é encontrada como cassetes nos integrons de classe 1 (94,97-101) Porém, os genes da MβL tipo IMP também podem ser encontrados em integrons de classe 2 e 3 (60,102,103).

Dentre os tipos de MβLs adquiridas, as de maior importância para a disseminação epidemiológica e relevância clínica são as enzimas IMP, VIM, SPM e NDM, especialmente a NDM-1, que constitui preocupação devido à sua tendência à disseminação intercontinental. Os outros tipos de MβLs adquiridas, SIM-1, GIM-1, AIM-1, KHM-1 e DIM-1, têm uma menor taxa de disseminação e impacto clínico quando comparadas a esta última (76).

Durante muitos anos, a ocorrência de isolados produtores de IMP-1, a primeira MβL adquirida reportada, oriunda de um isolado clínico de *Serratia marcescens* no Japão (89), permaneceu restrita ao seu país de origem. Entretanto, posteriormente, a IMP-1, bem como outras imipemenases, foram detectadas em diferentes microrganismos como *Acinetobacter* spp., *P. aeruginosa* e *K. pneumoniae* isolados de diferentes regiões geográficas (98,100,104-111). A maior prevalência de MβL tipo IMP na América Latina é encontrada no Brasil e na Argentina, onde existe uma alta ocorrência de isolados de *Acinetobacter* spp. multirresistentes (60,112). O primeiro *A. baumannii* brasileiro produtor de MβL do tipo IMP foi isolado de uma paciente internada no Hospital São Paulo (113). Posteriormente, uma *P. aeruginosa* com resistência a todos os beta-lactâmicos, inclusive imipenem e meropenem, foi isolada no Hospital de Base de Brasília, em 2002, e apresentava uma nova variante de IMP, designada IMP-16 (114). Existem, até então descritas, 24 variantes de IMP, sendo que em *A. baumannii* as MβL do tipo IMP reportadas são IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, IMP-8 e IMP-11 (115,116).

Relatos de oxacilinases pertencentes à classe D de Ambler têm aumentado em *A. baumannii* (73,117). Todas as beta-lactamases da classe D hidrolisam significantemente amino- e carboxipenicilinas (117) e podem aumentar a resistência aos carbapenêmicos em *A. baumannii* (118). Essas oxacilinases usualmente não são inibidas por ácido clavulânico, tazobactam e sulbactam, entretanto sua atividade pode ser inibida *in vitro* por cloreto de sódio, propriedade não demonstrada pelas outras classes de beta-lactamases, tornando-a útil para sua identificação (117).

As carbapenemases da classe D possuem 150 variantes descritas até o momento, sendo a mais diversa entre as quatro classes de beta-lactamases (117). Essas enzimas

são subdivididas em quatro tipos: estreito espectro (NS-OXAs), amplo espectro (ES-OXAs), hidrolizantes de carbapenêmicos (CHDLs), todas adquiridas; e uma de ocorrência natural (117). Os genes que codificam para essas enzimas estão principalmente associados com integrons de classe 1, sequências de inserção e transposons (117).

As NS-OXAs não estão muito relacionadas com isolados de *A. baumannii*, porém existem relatos deste microrganismo apresentando as enzimas OXA-3, OXA-20 e OXA-37 na Colômbia, França e Itália, e na Espanha, respectivamente (15,119-123). As ES-OXAs, até o momento, foram identificadas principalmente em *P. aeruginosa* (117).

A maioria das CHDLs descritas está presente em *A. baumannii* (117). A primeira oxacilinase adquirida com atividade de carbapenemase foi detectada em um isolado de *A. baumannii* proveniente da Escócia, em 1985, denominada OXA-23 (também conhecida como Ari-1) (124). A OXA-23 tem atividade hidrolítica moderada contra carbapenêmicos e aumenta a resistência a estas drogas em *A. baumannii* (118). Surtos causados por *Acinetobacter* produtor de OXA-23 têm sido reportados em várias regiões do mundo (125-128), inclusive no Brasil, onde foi reportado um surto de *A. baumannii* resistente aos carbapenêmicos e produtor de OXA-23 em dois hospitais de Curitiba, correspondendo ao primeiro relato da presença de oxacilinase adquirida no país (126). Em Porto Alegre, a primeira descrição de *A. baumanii* produtor de OXA-23 ocorreu em 2009 (63). E, em um estudo recente, um grupo de pesquisadores desta mesma cidade reportou a presença de três isolados de *A. baumannii* coletados de águas residuais de diferentes Hospitais, os quais foram positivos para o gene $bla_{\text{OXA-23}}$ *like* e apresentaram perda de suscetibilidade a todos os antimicrobianos testados (129).

As oxacilinases são divididas em oito subgrupos, dos quais quatro foram identificados em *A. baumannii*: OXA-23 *like* (OXA-23, OXA-27 e OXA-49); OXA-24 *like* (OXA-25, OXA-26, OXA-40); OXA-58 (variantes OXA-96 e OXA-97) e OXA-51 *like* (118,130,131), sendo que os três primeiros correspondem a CHDLs (117). Outros grupos menores de CHDLs foram identificados, como OXA-72 e a recentemente descrita OXA-143, a qual foi identificada em um isolado clínico brasileiro de *A. baumannii* (117,132).

Dentre as beta-lactamases de classe D de ocorrência natural, foram descritas a OXA-51 e OXA-69, ambas presentes em *A. baumannii*, constituindo o subgrupo OXA-51/OXA-69, que apresenta fraca similaridade com outras oxacilinases conhecidas (117).

Os genes que codificam as beta-lactamases *bla*$_{\text{OXA-51}}$ *like* estão presentes no cromossomo em todos os isolados de *A*. *baumannii* e não contribuem significantemente para os padrões de resistência natural observados em *A*. *baumannii*. Genes codificando variantes de OXA-51/OXA-69 (OXA-64, OXA-65, OXA-66, OXA-68, OXA-70, OXA-71, OXA-78, OXA-79, OXA-80, OXA-82, OXA-83, OXA-92, OXA-98, OXA-106, OXA-107, OXA-108, OXA-109, OXA-110, OXA-111 e OXA-112) (61,133) foram identificados a partir de uma grande coleção de isolados de *A*. *baumannii* de várias áreas geográficas (61,134), inclusive entre *A*. *baumannii* resistentes aos carbapenêmicos (133,135,136). Estudos recentes descreveram que as sequências de inserção ISA*ba1* e ISA*ba9* podem estar localizadas *upstream* ao gene *bla*$_{\text{OXA-51}}$*like* em isolados de *A. baumannii* resistentes aos carbepenêmicos, potencializando a expressão de OXA-51 ou OXA-51/OXA-69 *like*, diminuindo, assim, a suscetibilidade à ceftazidima e aos carbapanêmicos (137-139). Como estes genes são aparentemente ubíquos e restritos ao *A*. *baumannii*, tem sido utilizada a identificação desta espécie baseando-se na detecção do *bla*$_{\text{OXA-51}}$-*like* (136).

Diante da resistência do *A. baumannii* aos carbapenêmicos, a polimixina, embora apresente alta toxicidade, tem sido a droga de escolha para o tratamento de isolados resistentes aos carbapenêmicos (140). Atualmente, amostras de *A. baumannii* MDR sensíveis apenas à polimixina B têm sido isoladas pelos laboratórios de microbiologia clínica na maior parte dos hospitais brasileiros e do mundo (141-144). No entanto, já tem sido relatada a resistência de *Acinetobacter* spp., inclusive *A*. *baumannii*, às polimixinas (141,145-148). Além da polimixina, a tigeciclina é um dos poucos antimicrobianos que possui atividade contra *A*. *baumannii* MDR, sendo utilizada tanto como monoterapia quanto como terapia combinada com imipenem e/ou colistina (149,150). Essa droga possui boa atividade bacteriostática para amostras de *A. baumannii*, incluindo aquelas que são resistentes ao imipenem (10,151). Porém, já existem relatos de amostras de *A*. *baumannii* MDR resistentes também à tigeciclina (146,152,153). Um grupo de pesquisadores reportou recentemente, o aparecimento de amostras de *A*. *baumannii* extremamente resistentes (denominadas XDR), isolados de pacientes internados em UTIs de um Centro Médico. Estas amostras mostraram-se resistentes a todas as drogas antimicrobianas testadas, incluindo polimixina B, colistina e tigeciclina (146). Nestas situações, a terapia combinada de antimicrobianos parece ser a alternativa mais apropriada para o combate de *A*. *baumannii* MDR. Combinações

sugeridas incluem imipenem e amicacina, colistina e rifampina, polimixina B e rifampicina, e a associação de imipenem, rifampina e colistina (8,144).

1.2 Objetivos

1.2.1 Objetivo Geral

Este trabalho teve como objetivo detectar e determinar a resistência a drogas antimicrobianas de isolados nosocomiais de *A. baumannii*.

1.2.2 Objetivos Específicos

1.2.2.1 Isolar e identificar *A. baumannii* a partir de amostras ambientais e das luvas dos profissionais que trabalham na UTI Geral Adulto do Hospital São Lucas da PUCRS através de métodos fenotípicos e genotípicos;

1.2.2.2 Determinar a resistência a drogas antimicrobianas de *A. baumannii* isolados de ambiente e de luvas, bem como de isolados clínicos obtidos no laboratório do mesmo hospital, através da técnica de difusão de discos em agar;

1.2.2.3 Determinar a concentração inibitória mínima para o imipenem e polimixina B em isolados de *A. baumannii*;

1.2.2.4 Caracterizar isolados de *A. baumannii* quanto à produção de metalo-beta-lactamases através da técnica de aproximação de disco;

1.2.2.5 Detectar o gene bla_{IMP} nos isolados clínicos e não clínicos de *A. baumannii*;

1.2.2.6 Detectar o gene $bla_{\text{OXA-23}}$ em isolados de *A. baumannii* resistentes aos carbapenêmicos;

1.2.2.7 Detectar a presença de integrons das classes 1, 2 e 3 através da PCR em isolados de *A. baumannii*.

Capítulo 2

2.1 Artigo Científico

Detection and antimicrobial resistance determination of *Acinetobacter baumannii* nosocomial isolates

Artigo científico submetido ao *Journal of Infection*, publicado pela Elsevier

Fator de impacto: 3.805

Silvia Dias de Oliveira

De: ees.yjinf.0.165241.b7685364@eesmail.elsevier.com em nome de Journal of Infection **Enviada:** qui 5/1/2012 08:34
Para: Silvia Dias de Oliveira
Cc:
Assunto: A manuscript number has been assigned: YJINF-D-12-00012
Anexos:

Journal of Infection
Ref: YJINF-D-12-00012
Title: DETECTION AND ANTIMICROBIAL RESISTANCE DETERMINATION OF Acinetobacter baumannii NOSOCOMIAL ISOLATES
Authors: Otávio H Raro; Stephanie W Gallo; Luciana R Alcântara; Ana Maria Sandri; Carlos Alexandre S Ferreira; Sílvia Dias de Oliveira, Ph.D.
Article Type: Full Length Article

Dear Dr. Sílvia Dias de Oliveira,

Your submission entitled "DETECTION AND ANTIMICROBIAL RESISTANCE DETERMINATION OF Acinetobacter baumannii NOSOCOMIAL ISOLATES" has been assigned the following manuscript number: YJINF-D-12-00012.

You may check on the progress of your paper by logging on to the Elsevier Editorial System as an author. The URL is http://ees.elsevier.com/yjinf/.

Thank you for submitting your work to this journal. Please do not hesitate to contact me if you have any queries.

Kind regards,

D. Jones
Administrative Support Agent [17-Jan-11]
Journal of Infection

****
For any technical queries about using EES, please contact Elsevier Author Support at support@elsevier.com
Global telephone support is available 24/7:
For The Americas: +1 888 834 7287 (toll-free for US & Canadian customers)
For Asia & Pacific: +81 3 5561 5032
For Europe & rest of the world: +353 61 709190

DETECTION AND ANTIMICROBIAL RESISTANCE DETERMINATION OF *Acinetobacter baumannii* NOSOCOMIAL ISOLATES

Otávio H. F. Raro[a], Stephanie W. Gallo[a], Luciana R. Alcântara[b], Ana M. Sandri[b], Carlos A. S. Ferreira[a], Sílvia D. Oliveira[a,*]

[a]*Laboratório de Imunologia e Microbiologia, Faculdade de Biociências, PUCRS, Porto Alegre, RS, 90619-900, Brazil*

[b]*Hospital São Lucas, PUCRS, Porto Alegre, RS, 90610-000, Brazil*

Running title: *Acinetobacter baumannii*: detection and resistance

[*]Corresponding author:

Faculdade de Biociências, Pontifícia Universidade

Católica do Rio Grande do Sul (PUCRS),

Av. Ipiranga 6681, 90619-900, Porto Alegre, Brasil

Tel.: +55-51-33534953; fax: +55-51-33203568

E-mail address: silviadias@pucrs.br

Summary

Objectives: The aim of this study was to evaluate the presence of *A. baumannii* in an ICU environment and to characterize the antimicrobial drug resistance of the clinical and non-clinical isolates obtained.

Methods: 886 non-clinical samples were collected from an ICU. Antimicrobial resistance was determined in *A. baumannii* nosocomial isolates identified by PCR using 16S rDNA and $bla_{\text{OXA-51}}$ genes as targets. The presence of integrons, $bla_{\text{OXA-23}}$ and bla_{IMP} was evaluated by PCR.

Results: *A. baumannii* was isolated in 9.6% of samples collected from an ICU environment, and 46 clinical isolates were sent by the laboratory of the same hospital. The majority of isolates was MDR, and all isolates harboring integrons were MDR. High rates of resistance to carbapenems were detected in both clinical and non-clinical isolates. Among isolates with reduced susceptibility to carbapenems, all presented $bla_{\text{OXA-23}}$, and 41.4% non-clinical and 54% clinical isolates carried the bla_{IMP}. The resistance to polymyxin B was detected in 19.6% and 24.7% non-clinical and clinical isolates, respectively.

Conclusion: All *A. baumannii* isolates presented high resistance rates to the drugs tested. However, the resistance in the non-clinical isolates is of great concern because it can indicate the hospital environment as possible reservoir of resistant *A. baumannii*.

KEYWORDS: *Acinetobacter baumannii*; Antimicrobial drug resistance; β-lactamases; Hospital infections; Carbapenems

Introduction

Acinetobacter baumannii is an important opportunistic pathogen commonly associated with nosocomial infections, especially in patients hospitalized in intensive care units (ICUs). This pathogen is renowned for its ability to survive in the environment in dry conditions for prolonged periods, and can spread through air, water droplets over short distances, skin scaling of colonized patients (1), and the hands of workers in hospitals, which is the most common mode of transmission (2).

The treatment of infections caused by *A. baumannii* is difficult to be performed due to the multidrug-resistance (MDR) observed in many strains of this species (3). The emergence of MDR *A. baumannii* has been attributed to its ability to rapidly acquire resistance determinants, being very susceptible to horizontal gene transfer (4,5). Indeed, this pathogen may carry class 1, 2 and 3 integrons containing antimicrobial resistance genes (6,7), explaining, at least partially, its rapid positive response to antibiotic selective pressure. In this sense, carbapenems have been widely used to treat MDR *A. baumannii* infections (8,9), but isolates resistant to this drug have been described worldwide (10-16). Carbapenems resistance is mainly derived from β-lactamase production (17-20), but it is also found due to loss of permeability of the external membranes, change in the affinity of penicillin binding proteins (PBPs), and, rarely, by the overexpression of efflux pumps (21-23). Among metallo-β-lactamases (MβL), the imipenemase (IMP) is of great importance to epidemiologic dissemination and clinical relevance (24). The oxacillinases, Ambler class D β-lactamases, have been widely reported in *A. baumannii* (25,26), mainly the acquired OXA-23, OXA-24/40 and OXA-58, and OXA-51, the last one being intrinsically located in the chromosome (27-29).

The OXA-23, an important representative of this group, has been associated with various infection outbreaks in different parts of the world (30), and the OXA-51 does not seem to contribute substantially to the standards of carbapenem resistance observed in *A. baumannii* (25), although this gene has been used as a target to identify *A. baumannii* by PCR (31-35).

The treatment of carbapenem resistant *A. baumannii* infections has been performed with polymyxins (36), since MDR *A. baumannii* sensitive only to polymyxin B has been isolated worldwide (37-40). However, polymyxins resistant *A. baumannii* isolates have already been reported (14,37,41-43).

Therefore, the aim of this study was to evaluate the presence of the *A. baumannii* from an ICU environment and to characterize the antimicrobial resistance of the isolates obtained, as well as of the isolates from patients in ICU of the same hospital in which environmental samples were collected.

Materials and methods

Bacterial strains

From March to November 2010, a total of 886 samples were collected from a nosocomial environment (n=858) and from gloves (n=28) of the healthcare workers of an adult ICU of a 603-bed university hospital in Porto Alegre, a city located in Southern Brazil. Floor samples (n=33) were collected with a “drag swab” soaked in 0.1% saline peptone; the gloves were collected and transported in sterilized plastic bag, and the remaining samples were collected with a swab soaked in 0.1% saline peptone. The

Department of Microbiology of the Clinical Pathology Laboratory of the hospital sent 46 strains of *A. baumannii* from clinical specimens isolated in the same period.

Isolation and identification

The swabs collected from the hospital environment were introduced in 3 mL of BHI broth (Himedia) and incubated at a 150-rpm agitation at 37ºC for 24 h according to Baumann (44), and spread on MacConckey agar (Oxoid). An aliquot of 5 mL of nutrient broth was added to the internal part of each glove, homogenized and incubated for 5 min at room temperature. After, an aliquot of 100 µL was spread on blood agar (Himedia) and incubated at 37°C for 24h. An aliquot of 100 µL of saline solution obtained from the floor sample was spread on blood agar and incubated at 37°C for 24h. The colonies were preliminary screened by morphology and accordingly with the results were submitted to the characterization by oxidase test (Laborclin) and triple sugar iron (TSI) test (Oxoid). All tests were standardized using the reference culture *A. baumannii* ATCC 19606.

Isolates presenting a biochemical profile compatible with a presumptive identification of *Acinetobacter* spp. were submitted to DNA extraction using guanidine isothiocyanate according to Rademaker and De Brujin (45). The purified DNA was used as template for the PCR using the 16S rDNA (46) and $bla_{\text{OXA-51}}$ genes as targets to identify *Acinetobacter* spp. and *A. baumannii*, respectively. The primers targeting the oxacilinase OXA-51 were designed in this study to amplify a fragment of 347 bp specific for *A. baumannii* (5'GACGGGCAAAAAAGGCTA3' and 5'GGGTCTACATCCCATCCC3'). The PCR amplifications were performed in a final volume of 25 µL containing 1 µL of target DNA, 0.2 mM of each deoxynucleoside

triphosphate (dNTP), 2.5 μL of 10X PCR buffer (Invitrogen), 1.5 mM of $MgCl_2$, 0.2 U of *Taq* DNA polymerase (Invitrogen) and 20 ρmol of each primer. The amplification conditions used were: initial denaturation of 95°C by 5 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min and extension at 72°C for 1 min and 30 s, with a final extension at 72°C for 10 min. In order to determine the specificity of the designed primers to $bla_{\text{OXA-51}}$, 9 isolates identified presumptively as *Acinetobacter* spp. by biochemical tests were used. These strains and *A*. *baumannii* ATCC 19606 were analyzed by PCR in parallel to the biochemical identification with API 20 NE system. Also, two amplification products from OXA-51 were purified with ammonium acetate and submitted to sequencing in an ABI 3130 XL Genetic Analyzer (Applied Biosystems, Lincoln Centre Drive Foster City, USA) automated DNA sequencer.

Antimicrobial susceptibility testing

The antimicrobial resistance was performed according to the CLSI guidelines (47) to the disk diffusion technique to all *A*. *baumannii* isolates. The antimicrobials tested were: amikacin (AMI), ampicillin-sulbactam (AMS), aztreonam (ATM), cefepime (CPM), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), ciprofloxacin (CIP), doxycycline (DX), gentamicin (GEN), imipenem (IPM), levofloxacin (LVX), meropenem (MEM), minocycline (MIN), piperacillin-tazobactam (PTZ), trimethoprim-sulfamethoxazole (COT), tetracycline (TET), ticarcillin-clavulanic acid (TCC), and tobramycin (TOB). MDR was defined as the absence of susceptibility to three or more classes of antimicrobials (48-53).

The susceptibility to polymyxin B of all *A*. *baumannii* isolates was determined by minimum inhibitory concentration (MIC) using microdilution test and interpreted according to the CLSI guidelines (54). The resistance to imipenem in carbapenem resistant isolates in disk diffusion test was also evaluated by the determination of MIC. Disk diffusion test and MIC determination were performed using the reference cultures *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 as quality control.

Metallo-β-lactamase phenotypic detection

A disc-approximation test (55) using imipenem (Sensifar) and ceftazidime (Sensifar) as substrates and EDTA (Nuclear) as inhibitor was employed to detect MβL production. *P. aeruginosa* IMP-1 positive was used as control.

Detection of antimicrobial resistance determinants

The presence of the genes bla_{IMP} and bla_{OXA-23}, which belong to Ambler class B and D carbapenemases, respectively, was evaluated by PCR in isolates that showed reduced susceptibility to carbapenems. Previously described primers (56) were used to detect bla_{OXA-23}, and the primer pair targeting the MβL IMP was designed in this study (5'AGATACTGAAAAGTTAGTC3' and 5'TTGRAACWACCAGTTTTGC3'), amplifying a fragment of 424 bp. These primers were used to identify IMP, since they are able to amplify fragments from several IMPs (IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, IMP-7, IMP-8, IMP-10, IMP-11, IMP-15, IMP-16, IMP-18, IMP-19, IMP-20, IMP-21, IMP-22, IMP-24, IMP-25, IMP-26, IMP-27 and IMP-29). The class 1, 2 and 3 integrons were detected by PCR using a degenerate primer pair to detect genes from integrases *intI1*, *intI2* and *intI3* (57).

PCR amplifications were carried out in 25 µL volumes containing 1 µL of target DNA, 0.2 mM of each dNTP, 2.5 µL of 10X PCR buffer (Invitrogen), 0.2 U of *Taq* DNA polymerase (Invitrogen) and 20 ρmol of each primer. The $MgCl_2$ concentration and the amplification conditions to detect bla_{OXA-23} and integrons were used according to previously described (56,57). The same conditions were used to amplify the bla_{IMP} gene, except for altering $MgCl_2$ to 2 mM, the annealing temperature to 47°C, and the number of cycles to 30. The amplifications were carried out in a MiniCycler™ (MJ

Research) and the amplification products were analyzed by electrophoresis in agarose gels stained with 0.5 μg/mL ethidium bromide and visualized under UV radiation. Two amplification products from integrons and one from OXA-23 were purified and sequenced as described above. Searches and alignments for the nucleotide sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

Statistical analysis

Data were analyzed and compared by χ^2 test or Fisher's exact test, as appropriate. A *p* value <0.05 was considered statistically significant. When necessary, the data were analyzed and compared by Kappa coefficient test, using an Odds Ratio (OR) with 95% of confidence intervals (95% CI). To analyze the difference of resistance in all drugs tested within each source (non-clinical or clinical), the drugs were grouped in four clusters based on resistance rates (0-25%, 25%-50%, 50-75%, and 75%-100%) and evaluated by Friedman or Cochran tests.

Results

A. baumannii was detected in 9.6% (85) of the 886 samples collected from the hospital ICU environment and gloves, referred as non-clinical isolates. Regarding the *A. baumannii* positive samples, 4.7% (4) were isolated from floor and 2.3% (2) from gloves. Other strains (79) were isolated mainly from bed sides (23.5%), bidet (10.6%), and supplying balloon (9.4%). However, it was possible to isolate this microorganism in many other places of ICU rooms, such as monitor of mechanical ventilator, valves (of oxygen, air, and vacuum), infusion pump, door handles, stethoscope, purge, door, mask of ventilation, table for nursing staff support, chair, food delivery car, car of instruments, car for patient care, biological waste, pressure gauge, feeding table, heart rate monitor, staff clipboard (for information about patients), surface of the medical preparation car and serum support apparatus.

All 85 non-clinical and 46 clinical isolates biochemically compatible with *Acinetobacter* spp. were positives in 16S rDNA and $bla_{\text{OXA-51}}$ PCR tests (Fig. 1). The isolates used to standardize the $bla_{\text{OXA-51}}$ detection that harbored this gene were also identified as *A. baumannii* in the API 20 NE system. Moreover, the analysis of two $bla_{\text{OXA-51}}$ amplification product sequences showed at least 98% of similarity with other *A. baumannii* OXA-51 sequences deposited in GenBank.

Antimicrobial susceptibility of *A. baumannii* isolates to 19 antimicrobial agents is summarized in Table 1. The reduced susceptibility rates, i.e., resistance or intermediate resistance in the disk-diffusion test, to the majority of the drugs tested showed no difference between clinical and non-clinical isolates ($p>0.05$), with the exceptions of ceftazidime and amikacin. Furthermore, both groups of isolates showed the highest resistance rates to the same five drugs (aztreonam, ceftriaxone, cefotaxime,

cefepime and ticarcillin-clavulanic acid), as well as the lowest resistance rates to doxycycline and minocycline (Table 1).

The analyses of reduced susceptibility rates in all drugs tested within each source isolates (non-clinical or clinical) are shown in Table 2, and both analyses showed significant differences ($p<0.0001$) between all clusters. The results indicate a great similarity between the non-clinical and clinical clusters, with the exceptions of tetracycline (non-clinical and clinical isolates were grouped in cluster II and cluster I, respectively), levofloxacin and ceftazidime (both grouped in cluster III among non-clinical isolates, and grouped in cluster IV among clinical isolates). However, the non-clinical isolates were resistant to more antimicrobial classes than clinical isolates ($p<0.05$).

In this study, 98.8% (84) non-clinical and 97.8% (45) clinical isolates were found as MDR. Only one strain isolated from floor was susceptible to all drugs tested. From the non-clinical isolates, 50 different resistance profiles were obtained, whereas 33 were obtained from clinical isolates. The most common profiles found between all isolates included the resistance to at least 14 drugs, being the most prevalent profile (11.8%) among non-clinical isolates characterized by the reduced susceptibility to the following 17 drugs: TCC-IPM-LVX-MEM-CAZ-TET-TOB-CTX-CIP-AMI-CRO-CPM-PTZ-ATM-GEN-AMS-COT. From the clinical isolates, there were two more prevalent profiles (10.9% each one), one of them presenting reduced susceptibility to the same drugs found in the predominant profile of non-clinical isolates, except for tetracycline; the other prevalent profile presented two differences (absence of resistance to amikacin and tetracycline).

High reduced susceptibility rates to carbapenems were detected in 82.3% (70) of non-clinical and 80.4% (37) of clinical isolates by disk diffusion and MIC determination to imipenem, and no difference was found between the rates of reduced susceptibility to imipenem and meropenem in the two groups of isolates (p>0.05).

MβL production was evaluated in all carbapenems resistant isolates. In the non-clinical group, 41.4% of isolates were MβL producers, being 18.6% positives for ceftazidime, 15.7% for imipenem and 7.1% for both. Among the clinical isolates, 51.3% were MβL producers, being 10.8% positives for ceftazidime, 29.7% for imipenem and 10.8% for both. No difference (p>0.05) between non-clinical and clinical isolates was found analyzing the data obtained by the two tests or by each substrate.

The presence of bla_{IMP} gene was detected in 41.4% (29) and 54% (20) of non-clinical and clinical isolates presenting reduced susceptibility to carbapenems, respectively (Table 3). Also, it was detected bla_{IMP} gene in 5 non-clinical and 3 clinical carbapenems susceptible isolates. There was no significant difference in the presence of this target gene between the two groups of isolates (p>0.05). Among the 49 isolates carrying bla_{IMP}, 73.5% (36) showed to be MβL producers when tested with at least one substrate, whereas the proportion reduced to 61.2% (30) when using only imipenem. The analysis by Kappa coefficient considering the presence of bla_{IMP} as gold standard demonstrated that this gene was consistently associated with MβL production. Coexistence of bla_{IMP} and integrons was detected in 26.7% (35) strains. A total of 67.1% (57) non-clinical and 69.6% (32) clinical isolates showed to harbor *intI* integrase genes, and all 89 isolates harboring integrons were MDR. Sequencing of two *intI* amplicons identified both as *intI*2. All *A. baumannii* isolates resistant to carbapenems harbored $bla_{\text{OXA-23}}$ gene (Table 3).

The resistance to polymyxin B found in non-clinical isolates (19.6%) was not significantly different (p=0.6523) from that observed in clinical isolates (24.7%). Among the clinical *A. baumannii* isolates, 71.7% (33) were from patients treated with polymyxin B and 9.1% (3) of them showed resistance to this drug. It was observed significant association between treatment with polymyxin B and death, but there was no association between resistance to this drug and mortality.

Discussion

A. baumannii is an emerging pathogen that causes several nosocomial infections. Its ability to survive many months in the hospital environment and to readily acquire antimicrobial resistance has driven these bacteria to become an important concern for the medical community (2,9,58). In this study, we evaluated the presence of this microorganism in the ICU environment, as well as the status of antimicrobial resistance of the isolates, comparing to clinical isolates obtained in the same period and hospital. Therefore, a total of 886 samples were collected from environment and gloves of the ICU, and 9.6% of samples showed *A. baumannii*, which is an occurrence similar to those found by the majority of studies performed in ICU environments (59-62). The environmental isolates were recovered from different places of ICU rooms, including floor, as already reported (19,58,59,63,64). However, bed side was the most contaminated place, which indicates it as a probable environmental reservoir of *A. baumannii* in ICU.

Additionally to the ICU environment, the hands of healthcare workers are an important way of transmission of different pathogens (65-67), acting as a source of cross-infection and re-infection. Thus, in this study the inner side of gloves used by the

workers of the ICU was analyzed as an indicator of hand contamination, finding 7.4% of gloves contaminated with *A. baumannii*, while Young et al. did not find this microorganism in 50 gloves using a similar sampling method (68). The results described here present the occurrence of a large number of the ICU staff gloves contaminated with *A. baumannii*, even when comparing with other authors that investigated directly in hands using a similar number of samples (69). These results were unexpected, since the analysis of gloves is thought to underestimate the real prevalence of bacterial presence in hands. Güdücüoglu et al. (70) also showed high levels of occurrence of *A. baumannii* in gloves and hands, although the number of samples analyzed was significantly lower. It is also important to note that, as only internal part of gloves were sampled, we cannot evaluate the possible carriage of *A. baumannii* through the gloves, but it is possible to infer that this can occur as we found *A. baumannii* at different places of ICU.

The specific identification of *A. baumannii* was performed with a protocol developed in this study. Some authors had used the $bla_{\text{OXA-51}}$ gene as target to identify *A. baumannii* by PCR (31-35); however, a study has recently described the $bla_{\text{OXA-51}}$ gene in a clinical isolate of *Acinetobacter* 13TU with close identity of DNA sequence in the target region used in the previous studies (71-73). Therefore, to improve the *A. baumannii* detection, we designed a new primers pair to identify this pathogen, targeting another region of the $bla_{\text{OXA-51}}$ gene, resulting in the specific detection of this microorganism. Phenotypically, $bla_{\text{OXA-51}}$ has been associated with carbapenem resistance when there are ISA*ba1* and ISA*ba9* located upstream to this gene (33,74,75). So, although the presence of this gene can be associated with carbapenem resistance, we cannot assign the carbapenem resistance of the isolates found in this study to $bla_{\text{OXA-51}}$ because it was not evaluated the presence of these IS upstream to this gene.

All *A*. *baumannii* isolated from the environment, gloves and clinical samples from this study were analyzed with regard to susceptibility to antimicrobials, and the results indicated a great similarity between the non-clinical and clinical clusters, showing the importance of ICU environment as a reservoir of *A*. *baumannii*, which must be observed with considerable concern. The clinical and non-clinical isolates showed a high rate of antimicrobial multiresistance, especially when compared to other studies performed with clinical isolates that used the same criteria to define MDR (11,49-53,76), but we did not find any isolate presenting extensive drug resistance (XDR). The analysis of antimicrobial resistance of *Acinetobacter* spp. isolated from wastewater in the same hospital where we obtained our isolates showed only 21% of isolates as MDR (77). This discrepancy can be assigned to the difference in the source of isolates, in the period of time of the sampling (samples were collected 4 to 5 years before the sampling of this study), the definition of MDR adopted, and the species of *Acinetobacter*, since only 65% of the isolates from wastewater were *A*. *baumannii*.

The majority of non-clinical and clinical isolates showed to carry integrase genes, and all isolates harboring integrons were MDR, whereas the remaining 41 MDR integrons negative isolates probably possess the reduced susceptibility to the tested drugs mediated by resistance determinants not carried out by integrons. Many gene cassettes encoding resistance to a wide range of antibiotics had been associated to integrons (78-83), including acquired carbapenem resistance, which is the drug of choice for the treatment of *A*. *baumannii* infections. In this study, 80.9% of integron positive strains showed reduced susceptibility to carbapenems. Carbapenem resistance has become a great concern for the treatment of serious *Acinetobacter* infections. In our study, low susceptibility rates to carbapenems were detected, which has also been described by other authors both in clinical and non-clinical isolates; however, the other

analyses of ICU environment used a lower number of samples or had a lower prevalence of *A. baumannii* than those presented in this study (11,12,14,19,42,63,64,81,84,85).

The main carbapenem resistance mechanism in *A. baumannii* is acquired carbapenemase production of the class D carbapenemases, including OXA-23, or the IMP, VIM and SIM type metallo-β-lactamases (18,21). All *A. baumannii* isolates resistant to carbapenems showed to harbor the $bla_{\text{OXA-23}}$ gene, which has also been found by other authors in all isolates evaluated (85-89). This result also corroborates Ferreira et al. (77) results that found $bla_{\text{OXA-23}}$ gene in carbapenem resistant *A. baumannii* isolated from wastewater of the same hospital analyzed in this study. Such widespread prevalence can be due to horizontal transfer of $bla_{\text{OXA-23}}$, since it has been also identified on plasmids (90), but it has not been associated with integrons (46). On the other hand, the bla_{IMP} has been detected in integrons (82), and, although we did not analyze the presence of bla_{IMP} in integrons, 71.4% of our bla_{IMP} positive isolates also present integrons. Analyzing the two carbapemen resistance determinants investigated in this study, we found that 45.8% of the isolates with reduced susceptibility to carbapenem presented both bla_{IMP} and $bla_{\text{OXA-23}}$, what was also reported only, to date, by Koh et al., and in just 0.9% carbapenem resistant *A. baumannii* isolates (91).

The presence of bla_{IMP} in *A. baumannii* isolates have been reported worldwide (3,92-96), and the prevalence of this metallo-β-lactamase is discrepant among reports. In our study, the bla_{IMP} gene was detected in 41.4% and 54% of non-clinical and clinical isolates presenting reduced susceptibility to carbapenems, rates similar to those were reported by other authors in clinical isolates (3,84). However, the majority of the studies that evaluated the presence of bla_{IMP} described a low prevalence of the imipenemases encoded by this gene (11,62-64,81,94-96). bla_{IMP} was also detected in five non-clinical

and three clinical carbapenems susceptible isolates that were resistant to cephalosporin and/or penicillins, probably due to a broad substrate specificity of these imipenemases (24).

Among the isolates carrying bla_{IMP}, 73.5% were MβL producers when tested with at least one substrate, presenting a good correlation between the tests; however, false negatives results were found. Overall, the disc approximation test, even using both substrates and both inhibitors, raised contradictory results and presented limited reproducibility (77,97-99), not being an accurate way to determine MβL production.

In recent years, clinical isolates of *A. baumannii* resistant to polymyxin B have been described; however the rates of resistance showed some variation among the studies (14,41-43). The rates observed in this study were similar to those already described, but the presence of *A. baumannii* resistant to polymyxin B in the environment in similar levels of the clinical isolates raises great concern, since the environment can be a source of contamination by *A. baumannii* resistant to the drug of last choice for treatment of infections caused by this microorganism.

Furthermore, our results indicate the possibility of minocycline and doxycycline to be therapeutic options to treatment of CRAB infections, even those caused by polymyxin B resistant isolates. Similar results had already been described by other authors that found minocycline and doxycycline as effective antimicrobial drugs against *Acinetobacter* spp., since 80% to 90% of analyzed isolates were susceptible to this antibiotic (100-102). Moreover, minocycline has been used successfully for the treatment of wound infections and ventilator-associated pneumonia caused by MDR *A. baumannii* (103,104).

Our data show that the isolates present high resistance against the antimicrobial drugs tested. Additionally, they can carry resistance genes that probably are harbored in mobile genetic elements, especially integrons, what may facilitate horizontal transfer. These findings in non-clinical isolates are of great concern because they denote the ICU environment as a possible major reservoir of *A. baumannii* and the source for hospital generated infections with this microorganism.

Acknowledgements

Otávio H. F. Raro received a scholarship from CAPES, Brazil. We thank João Riboldi by statistical analyses support, the Clinical Pathology Laboratory of the Hospital São Lucas by sending *A. baumannii* from clinical specimens, and Infection Control Service and workers of the ICU by the help during the sampling on ICU.

References

1. Bernards AT, Frénay HM, Lim BT, Hendriks WD, Dijkshoorn L, van Boven CP. Methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*: an unexpected difference in epidemiologic behavior. *Am J Infect Control* 1998; 26:544-51, doi: 10.1053ic.1998.v26.a84555.

2. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 2007; 5:939-51.

3. Peymani A, Nahaei M-R, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, et al. High prevalence of metallo-beta-lactamase-producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. *Jpn J Infect Dis* 2011; 64:69-71.

4. Lorenz MG, Wackernagel W. Bacterial gene transfer by natural genetic transformation in the environment. *Microbiol Rev* 1994; 58:563-602.

5. Metzgar D, Bacher JM, Pezo V, Reader J, Döring V, Schimmel P, et al. *Acinetobacter* sp. ADP1: an ideal model organism for genetic analysis and genome engineering. *Nucleic Acids Res* 2004; 32:5780-90.

6. Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 2006; 4:608-20.

7. Cambray G, Guerout A-M, Mazel D. Integrons. *Annu Rev Genet* 2010; 44:141-66.

8. Kim B-N, Peleg AY, Lodise TP, Lipman J, Li J, Nation R, et al. Management of meningitis due to antibiotic-resistant *Acinetobacter* species. *Lancet Infect Dis* 2009; 9:245-55.

9. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clin Microbiol Rev* 2008; 21:538-82.

10. Naas T, Levy M, Hirschauer C, Marchandin H, Nordmann P. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. *J Clin Microbiol* 2005; 43:4826-9.

11. Carvalho KR, Carvalho-Assef APD, Peirano G, Santos LCGD, Pereira MJF, Asensi MD. Dissemination of multidrug-resistant *Acinetobacter baumannii* genotypes carrying bla(OXA-23) collected from hospitals in Rio de Janeiro, Brazil. *Int J Antimicrob Agents* 2009; 34:25-8, doi: 10.1016/j.ijantimicag.2008.12.009.

12. Tuon FF, Rymsza AM, Penteado-Filho SR, Pilonetto M, Arend LN, Levin AS. Should polymyxin be used empirically to treat infections in patients under high risk for carbapenem-resistant *Acinetobacter*? *J Infect* 2011; 62:246-9, doi: 10.1016/j.jinf.2011.01.005.

13. Quale J, Bratu S, Landman D, Heddurshetti R. Molecular epidemiology and mechanisms of carbapenem resistance in *Acinetobacter baumannii* endemic in New York City. *Clin Infect Dis* 2003; 37:214-20.

14. Kyoung Park Y. Extreme Drug Resistance in *Acinetobacter baumannii* Infections in Intensive Care Units, South Korea. *Emerg Infect Dis* 2009; 15:1325-1327.

15. Van Looveren M, Goossens H. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clin Microbiol Infect* 2004; 10:684-704.

16. Ho P-L, Ho AY, Chow K-H, Cheng VCC. Surveillance for multidrug-resistant *Acinetobacter baumannii*: a lesson on definitions. *Int J Antimicrob Agents* 2010; 36:469-71, doi: 10.1016/j.ijantimicag.2010.07.004.

17. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; 18:306-25.

18. Brown S, Amyes S. OXA (beta)-lactamases in *Acinetobacter*: the story so far. *J Antimicrob Chemother* 2006; 57:1-3.

19. Pournaras S, Markogiannakis a, Ikonomidis a, Kondyli L, Bethimouti K, Maniatis a N, et al. Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in an intensive care unit. *J Antimicrob Chemother* 2006; 57:557-61.

20. Martins AF, Kuchenbecker R, Sukiennik T, Boff R, Reiter KC, Lutz L, et al. Carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme: dissemination in Southern Brazil. *Infection* 2009; 37:474-6.

21. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 2006; 12:826-36.

22. Mussi MA, Relling VM, Limansky AS, Viale AM. CarO, an *Acinetobacter baumannii* outer membrane protein involved in carbapenem resistance, is essential for L-ornithine uptake. *FEBS lett* 2007; 581:5573-8, doi: 10.1016/j.febslet.2007.10.063.

23. Siroy A, Molle V, Lemaître-Guillier C, Vallenet D, Pestel-Caron M, Cozzone AJ, et al. Channel formation by CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2005; 49:4876-83.

24. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? *Lancet Infect Dis* 2011; 11:381-93.

25. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrob Agents Chemother* 2010; 54:24-38.

26. Bush K, Jacoby G a. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010; 54:969-76.

27. Bou G, Oliver A, Martínez-Beltrán J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. *Antimicrob Agents Chemother* 2000; 44:1556-61.

28. Walther-Rasmussen J, Høiby N. OXA-type carbapenemases. *J Antimicrob Chemother* 2006; 57:373-83.

29. Bou G, Cerveró G, Domínguez MA, Quereda C, Martínez-Beltrán J. Characterization of a nosocomial outbreak caused by a multiresistant *Acinetobacter baumannii* strain with a carbapenem-hydrolyzing enzyme: high-level carbapenem resistance in *A. baumannii* is not due solely to the presence of beta-lactamases. *J Clin Microbiol* 2000; 38:3299-305.

30. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. *J Antimicrob Chemother*; 63:55-9.

31. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006; 44:2974-6.

32. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006; 27:351-3.

33. Figueiredo S, Poirel L, Croize J, Recule C, Nordmann P. In vivo selection of reduced susceptibility to carbapenems in *Acinetobacter baumannii* related to ISAba1-mediated overexpression of the natural bla(OXA-66) oxacillinase gene. *Antimicrob Agents Chemother* 2009; 53:2657-9.

34. Chuang Y-C, Chang S-C, Wang W-K. High and increasing Oxa-51 DNA load predict mortality in *Acinetobacter baumannii* bacteremia: implication for pathogenesis and evaluation of therapy. *PloS One* 2010; 5(11):e14133.

35. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* 2010; 65:233-8.

36. Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2006; 50:2946-50.

37. Reis AO, Luz D a M, Tognim MCB, Sader HS, Gales AC. Polymyxin-resistant *Acinetobacter* spp. isolates: what is next? *Emerg Infect Dis* 2003; 9:1025-7.

38. Landman D, Bratu S, Kochar S, Panwar M, Trehan M, Doymaz M, et al. Evolution of antimicrobial resistance among *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in Brooklyn, NY. *J Antimicrob Chemother* 2007; 60:78-82.

39. Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. *Clin Microbiol Rev* 2008; 21:449-65.

40. Lim T-P, Tan T-Y, Lee W, Sasikala S, Tan T-T, Hsu L-Y, et al. In-vitro activity of polymyxin B, rifampicin, tigecycline alone and in combination against carbapenem-resistant *Acinetobacter baumannii* in Singapore. *PloS One* 2011; 6(4):e18485.

41. Ko KS, Suh JY, Kwon KT, Jung S-I, Park K-H, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *J Antimicrob Chemother* 2007; 60:1163-7.

42. Park YK, Choi JY, Jung S-I, Park K-H, Lee H, Jung DS, et al. Two distinct clones of carbapenem-resistant *Acinetobacter baumannii* isolates from Korean hospitals. *Diagn Microbiol Infect Dis* 2009; 64:389-95, doi: 10.1016/j.diagmicrobio.2009.03.029.

43. Park YK, Lee GH, Baek JY, Chung DR, Peck KR, Song J-H, et al. A single clone of *Acinetobacter baumannii*, ST22, is responsible for high antimicrobial resistance rates of *Acinetobacter* spp. isolates that cause bacteremia and urinary tract infections in Korea. *Microb Drug Resist* 2010; 16:143-9.

44. Baumann P. Isolation of *Acinetobacter* from soil and water. *J Bacteriol* 1968; 96:39-42.

45. Rademaker JLW, Bruijn FJ. Characterization and classification of microbes by REP-PCR genomic fingerprinting and computer-assisted pattern analysis. In DNA markers: protocols, applications, and overviews. New York: Caetano-Anollés G, Gresshoff PM, 1997.

46. Turton JF, Kaufmann ME, Glover J, Coelho JM, Warner M, Pike R, et al. Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. *J Clin Microbiol* 2005; 43:3074-82.

47. Clinical and Laboratory Standards Institute publication M100-S20 suggested grouping of US-FDA approved antimicrobial agents that should be considered for routine testing and reporting on nonfastidious organisms by Clinical Laboratories, 2010.

48. Paterson DL. The epidemiological profile of infections with multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clin Infect Dis* 2006; 43 Suppl 2(Suppl 2):S43-8.

49. Anthony KB, Fishman NO, Linkin DR, Gasink LB, Edelstein PH, Lautenbach E. Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline. *Clin Infect Dis* 2008; 46:567-70.

50. Eser OK, Ergin A, Tunçkanat F, Hasçelik G. In vitro activity of tigecycline as a therapeutic option against multidrug-resistant *Acinetobacter* spp. *New Microbiol* 2008; 31:535-42.

51. Keen EF, Murray CK, Robinson BJ, Hospenthal DR, Co E-MA, Aldous WK. Changes in the incidences of multidrug-resistant and extensively drug-resistant organisms isolated in a military medical center. *Infect Control Hosp Epidemiol* 2010; 31:728-32.

52. Wadl M, Heckenbach K, Noll I, Ziesing S, Pfister W, Beer J, et al. Increasing occurrence of multidrug-resistance in *Acinetobacter baumannii* isolates from four German University Hospitals, 2002-2006. *Infection* 2010; 38:47-51.

53. McCracken M, Mataseje LF, Loo V, Walkty A, Adam HJ, Hoban DJ, et al. Characterization of *Acinetobacter baumannii* and meropenem-resistant *Pseudomonas aeruginosa* in Canada: results of the CANWARD 2007-2009 study. *Diagn Microbiol Infect Dis* 2011; 69:335-41, doi: 10.1016/j.diagmicrobio.2010.10.030.

54. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. 2009;29.

55. Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-beta-lactamase-producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol* 2000; 38:40-3.

56. Donald HM, Scaife W, Amyes SG, Young HK. Sequence analysis of ARI-1, a novel OXA beta-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. *Antimicrob Agents Chemother* 2000; 44:196-9.

57. White PA, McIver CJ, Deng Y-mo, Rawlinson WD. Characterisation of two new gene cassettes, aadA5 and dfrA17. *FEMS Microbiol Lett* 2000; 182(2):265-9.

58. Joseph NM, Sistla S, Dutta TK, Badhe AS, Rasitha D, Parija SC. Role of intensive care unit environment and health-care workers in transmission of ventilator-associated pneumonia. *J Infect Dev Ctries* 2010; 4:282-91.

59. Fontana C, Favaro M, Minelli S, Bossa MC, Testore GP, Leonardis F, et al. *Acinetobacter baumannii* in intensive care unit: a novel system to study clonal relationship among the isolates. *BMC Infec Dis* 2008; 8:79.

60. Wilson APR, Smyth D, Moore G, Singleton J, Jackson R, Gant V, et al. The impact of enhanced cleaning within the intensive care unit on contamination of the near-patient environment with hospital pathogens: a randomized crossover study in critical care units in two hospitals. *Crit Care Med* 2011; 39:651-8.

61. Kraniotaki E, Manganelli R, Platsouka E, Grossato A, Paniara O, Palù G. Molecular investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii*, with characterisation of class 1 integrons. *Int J Antimicrob Agents* 2006; 28:193-9, doi: 10.1016/j.ijantimicag.2006.04.016.

62. Touati A, Achour W, Cherif A, Hmida HB, Afif FB, Jabnoun S, et al. Outbreak of *Acinetobacter baumannii* in a neonatal intensive care unit: antimicrobial susceptibility and genotyping analysis. *Ann Epidemiol* 2009; 19:372-8, doi: 10.1016/j.annepidem.2009.03.010.

63. Lin W-R, Lu P-L, Siu L-K, Chen T-C, Lin C-Y, Hung C-T, et al. Rapid control of a hospital-wide outbreak caused by extensively drug-resistant OXA-72-producing *Acinetobacter baumannii*. *Kaohsiung J Med Sci* 2011; 27:207-14.

64. Kulah C, Mooij MJ, Comert F, Aktas E, Celebi G, Ozlu N, et al. Characterisation of carbapenem-resistant *Acinetobacter baumannii* outbreak strains producing OXA-58 in Turkey. *Int J Antimicrob Agents* 2010; 36:114-8, doi: 10.1016/j.ijantimicag.2010.03.017.

65. Boyce JM, Potter-Bynoe G, Chenevert C, King T. Environmental contamination due to methicillin-resistant *Staphylococcus aureus*: possible infection control implications. *Infect Control Hosp Epidemiol* 1997; 18:622-7.

66. Dancer SJ. Importance of the environment in meticillin-resistant *Staphylococcus aureus* acquisition: the case for hospital cleaning. *Lancet Infect Dis* 2008; 8:101-13.

67. Sax H, Allegranzi B, Uçkay I, Larson E, Boyce J, Pittet D. "My five moments for hand hygiene": a user-centred design approach to understand, train, monitor and report hand hygiene. *J Hosp Infect* 2007; 67(1):9-21, doi: 10.1016/j.jhin.2007.06.004.

68. Young LS, Sabel AL, Price CS. Epidemiologic, clinical, and economic evaluation of an outbreak of clonal multidrug-resistant *Acinetobacter baumannii* infection in a surgical intensive care unit. *Infect Control Hosp Epidemiol* 2007; 28(11):1247-54.

69. Kohlenberg A, Brümmer S, Higgins PG, Sohr D, Piening BC, de Grahl C, et al. Outbreak of carbapenem-resistant *Acinetobacter baumannii* carrying the carbapenemase OXA-23 in a German university medical centre. *J Med Microbiol* 2009; 58:1499-507.

70. Güdücüoglu H, Durmaz R, Yaman G, Cizmeci Z, Berktas M, Durmaz B. Spread of a single clone *Acinetobacter baumannii* strain in an intensive care unit of a teaching hospital in Turkey. *New Microbiol* 2005; 28:337-43.

71. Donnarumma F, Sergi S, Indorato C, Mastromei G, Monnanni R, Nicoletti P, et al. Molecular characterization of *acinetobacter* isolates collected in intensive care units of six hospitals in Florence, Italy, during a 3-year surveillance program: a population structure analysis. *J Clin Microbiol* 2010; 48:1297-304.

72. Chen T-L, Lee Y-T, Kuo S-C, Hsueh P-R, Chang F-Y, Siu L-K, et al. Emergence and Distribution of Plasmids Bearing the blaOXA-51-like gene with an upstream ISAba1 in carbapenem-resistant *Acinetobacter baumannii* isolates in Taiwan. *Antimicrob Agents Chemother* 2010; 54:4575-81.

73. Lee Y-T, Turton JF, Chen T-L, Wu RC-C, Chang W-C, Fung C-P, et al. First identification of blaOXA-51-like in non-*baumannii Acinetobacter* spp. *J Chemother* 2009; 21:514-20.

74. Figueiredo S, Poirel L, Papa A, Koulourida V, Nordmann P. Overexpression of the naturally occurring blaOXA-51 gene in *Acinetobacter baumannii* mediated by novel insertion sequence ISAba9. *Antimicrob Agents Chemother* 2009; 53:4045-7.

75. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006; 258:72-7.

76. Joung MK, Kwon KT, Kang C-I, Cheong HS, Rhee J-young, Jung DS, et al. Impact of inappropriate antimicrobial therapy on outcome in patients with hospital-acquired pneumonia caused by *Acinetobacter baumannii*. *J Infect* 2010; 61:212-8, doi: 10.1016/j.jinf.2010.06.014.

77. Ferreira AE, Marchetti DP, De Oliveira LM, Gusatti CS, Fuentefria DB, Corção G. Presence of OXA-23-Producing Isolates of *Acinetobacter baumannii* in

Wastewater from Hospitals in Southern Brazil. *Microb Drug Resist* 2011; 17:221-7.

78. Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis* 1999; 18:761-70.

79. Rowe-Magnus DA, Mazel D. Resistance gene capture. *Curr Opin Microbiol* 1999; 2:483-8.

80. Yan Z-Q, Shen D-X, Cao J-R, Chen R, Wei X, Liu L-P, et al. Susceptibility patterns and molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* strains from three military hospitals in China. *Int J Antimicrob Agents* 2010; 35:269-73, doi: 10.1016/j.ijantimicag.2009.10.016.

81. Lin M-F, Chang K-C, Lan C-Y, Chou J, Kuo J-W, Chang C-K, et al. Molecular epidemiology and antimicrobial resistance determinants of multidrug-resistant *Acinetobacter baumannii* in five proximal hospitals in Taiwan. *Jpn J Infect Dis* 2011; 64:222-7, doi: 10.1016/j.kjms.2010.11.004.

82. Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, et al. Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. *Antimicrob Agents Chemother* 2007; 51:4022-8.

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

84. Sung JY, Kwon KC, Park JW, Kim YS, Kim JM, Shin KS, et al. Dissemination of IMP-1 and OXA type beta-lactamase in carbapenem-resistant *Acinetobacter baumannii*. *Korean J Lab Med* 2008; 28:16-23.

85. Niumsup PR, Boonkerd N, Tansawai U, Tiloklurs M. Carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 in Thailand. *Jpn J Infect Dis* 2009; 62:152-4.

86. Zhou H, Pi B-R, Yang Q, Yu Y-S, Chen Y-G, Li L-J, et al. Dissemination of imipenem-resistant *Acinetobacter baumannii* strains carrying the ISAba1 blaOXA-23 genes in a Chinese hospital. *J Med Microbiol* 2007; 56:1076-80.

87. Stoeva T, Higgins PG, Savov E, Markovska R, Mitov I, Seifert H. Nosocomial spread of OXA-23 and OXA-58 beta-lactamase-producing *Acinetobacter baumannii* in a Bulgarian hospital. *J Antimicrob Chemother* 2009; 63:618-20.

88. Dalla-Costa LM, Coelho JM, Souza HAPHM, Castro MES, Stier CJN, Bragagnolo KL, et al. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. *J Clin Microbiol* 2003; 41:3403-6.

89. Villegas MV, Kattan JN, Correa A, Lolans K, Guzman AM, Woodford N, et al. Dissemination of *Acinetobacter baumannii* clones with OXA-23 Carbapenemase in Colombian hospitals. *Antimicrob Agents Chemother* 2007; 51:2001-4.

90. Scaife W, Young HK, Paton RH, Amyes SG. Transferable imipenem-resistance in *Acinetobacter* species from a clinical source. *J Antimicrob Chemother* 1995; 36:585-6.

91. Koh TH, Tan TT, Khoo CT, Ng SY, Tan TY, Hsu L-Y, et al. *Acinetobacter calcoaceticus- Acinetobacter baumannii* complex species in clinical specimens in Singapore. *Epidemiol Infect* 2011; 21:1-4.

92. Da Silva GJ, Correia M, Vital C, Ribeiro G, Sousa JC, Leitão R, et al. Molecular characterization of bla(IMP-5), a new integron-borne metallo-beta-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. *FEMS Microbiol Lett* 2002; 215:33-9.

93. Riccio ML, Franceschini N, Boschi L, Caravelli B, Cornaglia G, Fontana R, et al. Characterization of the metallo-beta-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of bla(IMP) allelic variants carried by gene cassettes of different phylogeny. *Antimicrob Agents Chemother* 2000; 44:1229-35.

94. Lee M-F, Peng C-F, Hsu H-J, Chen Y-H. Molecular characterisation of the metallo-beta-lactamase genes in imipenem-resistant Gram-negative bacteria from a university hospital in southern Taiwan. *Int J Antimicrob Agents* 2008; 32:475-80, doi: 10.1016/j.ijantimicag.2008.07.009.

95. Jeong SH, Bae IK, Park KO, An YJ, Sohn SG, Jang SJ, et al. Outbreaks of imipenem-resistant *Acinetobacter baumannii* producing carbapenemases in Korea. *J Microbiol* 2006; 44:423-31.

96. Koo SH, Kwon KC, Cho HH, Sung JY. Genetic basis of multidrug-resistant *Acinetobacter baumannii* clinical isolates from three university hospitals in Chungcheong Province, Korea. *Korean J Lab Med* 2010; 30:498-506.

97. Uma Karthika R, Srinivasa Rao R, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, et al. Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of *Acinetobacter baumannii* from a South Indian tertiary care hospital. *J Med microbiol* 2009; 58:430-5.

98. Gupta V, Datta P, Chander J. Prevalence of metallo-beta lactamase (MBL) producing *Pseudomonas* spp. and *Acinetobacter* spp. in a tertiary care hospital in India. *J Infect* 2006; 52:311-4, doi: 10.1016/j.jinf.2005.08.013.

99. Noyal MJC, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. *Indian J Med Res* 2009; 129:707-12.

100. Draghi DC, Tench S, Dowzicky MJ, Sahm DF. Baseline in vitro activity of tigecycline among key bacterial pathogens exhibiting multidrug resistance. *Chemotherapy* 2008; 54:91-100.

101. Valentine SC, Contreras D, Tan S, Real LJ, Chu S, Xu HH. Phenotypic and molecular characterization of *Acinetobacter baumannii* clinical isolates from nosocomial outbreaks in Los Angeles County, California. *J Clin Microbiol* 2008; 46:2499-507.

102. Akers KS, Mende K, Yun HC, Hospenthal DR, Beckius ML, Yu X, et al. Tetracycline susceptibility testing and resistance genes in isolates of *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex from a U.S. military hospital. *Antimicrob Agents Chemother* 2009; 53:2693-5.

103. Griffith ME, Yun HC, Horvath LL, Murray CK. Minocycline Therapy for Traumatic Wound Infections Caused by the Multidrug-Resistant *Acinetobacter baumannii-Acinetobacter calcoaceticus* Complex. *Infect Dis Clin Pract* 2008; 16(1):16-19.

104. Wood GC, Hanes SD, Boucher BA, Croce MA, Fabian TC. Tetracyclines for treating multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *Intensive Care Med* 2003; 29:2072-6.

Table 1 Reduced susceptibility rates of non-clinical and clinical *A. baumannii* isolates to all tested drugs.

	Non-clinical isolates (n = 85)	Clinical isolates (n = 46)
	Reduced susceptibility (%)	
Antimicrobial drug		
MIN	8 (9.4)[a]	2 (4.3)[a]
TCC	76 (89.4)[a]	43 (93.5)[a]
IPM	68 (80)[a]	37 (80.4)[a]
LVX	62 (72.9)[a]	39 (84.8)[a]
MEM	70 (82.3)[a]	36 (78.3)[a]
CAZ	45 (52.9)[a]	35 (76.1)[b]
TET	34 (40)[a]	10 (21.7)[a]
DX	5 (5.9)[a]	0 (0)[a]
TOB	55 (64.7)[a]	28 (60.9)[a]
CTX	79 (92.9)[a]	45 (97.8)[a]
CIP	76 (89.4)[a]	41 (89.1)[a]
AMI	62 (72.9)[a]	23 (50)[b]
CRO	82 (96.5)[a]	45 (97.3)[a]
CPM	77 (90.6)[a]	44 (95.6)[a]
PTZ	73 (85.9)[a]	40 (86.9)[a]
ATM	81 (95.3)[a]	46 (100)[a]
GEN	61 (71.8)[a]	32 (69.6)[a]
AMS	62 (72.9)[a]	29 (63)[a]
COT	74 (87.1)[a]	36 (78.3)[a]

Different superscripts in the same line indicate significantly different values by χ^2 test (p<0.05).
MIN, minocycline; TCC, ticarcillin-clavulanic acid; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; CAZ, ceftazidime; TET, tetracycline; DX, doxycycline; TOB, tobramycin; CTX, cefotaxime; CIP, ciprofloxacin; AMI, amikacin; CRO, ceftriaxone; CPM, cefepime; PTZ, piperacillin-tazobactam; ATM, aztreonam; GEN, gentamicin; AMS, ampicillin-sulbactam; COT, trimethoprim-sulfamethoxazole.

Table 2 Clustering of drugs according to the reduced susceptibility rates for each *A. baumannii* source.

Cluster	Non-clinical isolates	Clinical isolates
I (0-25%)	MIN; DX	TET; MIN; DX
II (25-50%)	TET	-
III (50-75%)	TOB; LVX; GEN; CAZ; AMS; AMI	TOB; GEN; AMS; AMI
IV (75-100%)	TCC; PTZ; MEM; IPM; CTX; CRO; CPM; COT; CIP; ATM	TCC; PTZ; MEM; LVX; IPM; CTX; CRO; CPM; COT; CIP; CAZ; ATM

MIN, minocycline; TCC, ticarcillin-clavulanic acid; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; CAZ, ceftazidime; TET, tetracycline; DX, doxycycline; TOB, tobramycin; CTX, cefotaxime; CIP, ciprofloxacin; AMI, amikacin; CRO, ceftriaxone; CPM, cefepime; PTZ, piperacillin-tazobactam; ATM, aztreonam; GEN, gentamicin; AMS, ampicillin-sulbactam; COT, trimethoprim-sulfamethoxazole.

Table 3 Presence of *bla*$_{\text{OXA-23}}$ e *bla*$_{\text{IMP}}$ genes in imipenem resistant *A. baumannii* isolates at different minimum inhibitory concentration (MIC).

	MIC imipenem (µg/mL)				
Target gene	8	16	32	64	Total
bla$_{\text{OXA-23}}$	7	61	32	7	107*
bla$_{\text{IMP}}$	3	32	12	2	49

*This value also corresponds to all isolates that presented reduced susceptibility to carbapenems.

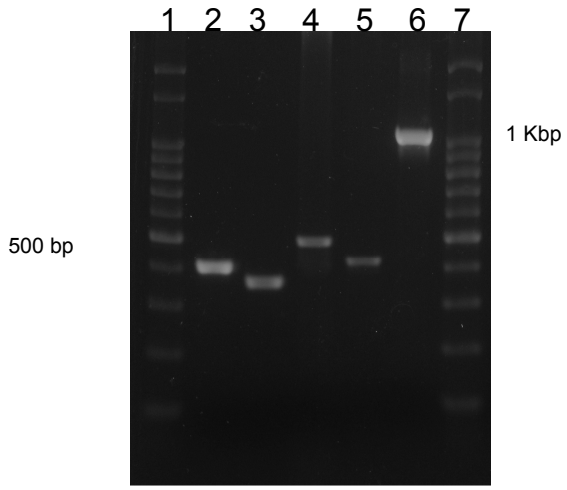

Figure 1 - Electrophoresis of amplicons using 16S rDNA (lane 2, 400 bp), *bla*$_{\text{OXA-51}}$ (lane 3, 347 bp), *intI* (lane 4, 491 bp), *bla*$_{\text{IMP}}$ (lane 5, 424 bp) and *bla*$_{\text{OXA-23}}$ (lane 6, 1065 bp) as target on 1.0% agarose gel stained with ethidium bromide. 100 bp molecular mass marker (Ludwig Biotecnologia) (lane 1 and 7).

Capítulo 3

Considerações Finais

3.1 Considerações finais

A determinação da presença de *A. baumannii* no ambiente hospitalar é de extrema importância, já que este microrganismo é o agente causal de infecções oportunistas em pacientes internados, especialmente em UTIs, tendo sido associado a diversos surtos no Brasil e no mundo (21,25,48,154-156).

A identificação presuntiva de *A. baumannii* nas amostras ambientais e de luvas foi realizada através de testes fenotípicos. Posteriormente, todos os isolados, clínicos e não clínicos, foram confirmados como *A. baumannii* através de PCR, tendo como alvos os genes 16S rDNA e $bla_{\text{OXA-51}}$. Para a realização da PCR tendo como alvo o gene $bla_{\text{OXA-51}}$, de ocorrência natural em *A. baumannii*, foi desenhado um par de oligonucleotídeos iniciadores específico para esta espécie, uma vez que o par de oligonucleotídeos iniciadores amplamente utilizado por outros autores (157-159) mostrou-se inespecífico para a detecção deste microrganismo, detectando também *Acinetobacter* 13TU (159).

Através da utilização do protocolo de detecção específica de *A. baumannii* desenvolvido neste estudo, foram identificados 85 (9,6%) isolados de *A. baumannii* em um total de 886 amostras ambientais e luvas coletadas da UTI. A ocorrência encontrada é semelhante às relatadas pela maior parte dos estudos realizados em ambiente de UTIs (48,160-162). As camas dos leitos, amostradas a partir de suas laterais, incluindo grades de proteção e botões para ajustar altura e posição dos pacientes, foi o local onde ocorreu o maior número (23,5%) de isolamentos de *A. baumannii*. Porém, *A. baumannii* também foi detectado em diversos outros locais, até mesmo onde o isolamento deste microrganismo seria menos esperado, como é o caso das mesas de entrega de refeições e de preparo de medicações, além de ter sido isolado da parte interna das luvas dos

técnicos de enfermagem, mostrando a ampla disseminação deste microrganismo na UTI avaliada.

A caracterização da resistência antimicrobiana em isolados de *A. baumannii* realizada neste estudo mostrou um grande número de isolados clínicos e não clínicos apresentando suscetibilidade reduzida a vários antimicrobianos testados, obtendo-se 98,5% de isolados MDR; entretanto, não foi detectado nenhum isolado de *A. baumannii* XDR. Também foi observado que as taxas de suscetibilidade reduzida detectadas nos isolados não clínicos foram muito similares àquelas apresentadas pelos isolados clínicos, demonstrando a importância do ambiente de uma UTI como um reservatório de patógenos clinicamente importantes que apresentam resistência a diversas drogas antimicrobianas, incluindo algumas importantes opções para o tratamento de infecções causadas por *A. baumannii*.

Muitos genes de resistência a drogas antimicrobianas podem ser carreados por integrons contendo elementos genéticos móveis (116,155,163-166). Desta forma, neste estudo foi verificada a presença de integrons utilizando os genes da integrase como alvos, tendo sido detectados em 67,9% dos isolados clínicos e não-clínicos. Dois isolados positivos para *int*I foram escolhidos aleatoriamente e submetidos ao sequenciamento. Ambos foram identificados como carreadores de integron classe 2, que não é a classe de integrons predominante entre *A. baumannii*, mas já foi demonstrada ser altamente prevalente neste microrganismo no estudo de Ramirez et al. (102). Entretanto, muitos estudos reportam que os integrons de classe 1 são os mais frequentes em *A. baumannii* MDR (46-48,122,166-168). Assim, a identificação da classe de integrons carreados pelos demais isolados de *A. baumanni* avaliados neste estudo serão posteriormente determinadas.

Os carbapenêmicos são as drogas de escolha para o tratamento de infecções causadas por *A. baumannii*, porém o aumento do número de relatos a respeito da crescente suscetibilidade reduzida desta bactéria a estes antimicrobianos (41,55,56), com os quais este estudo corroborou, tem se tornado uma grande preocupação na comunidade médica. Desta forma, com o objetivo de elucidar possíveis mecanismos responsáveis pela resistência aos carbapenêmicos nos isolados avaliados, inicialmente dois determinantes de resistência foram investigados, $bla_{\text{OXA-23}}$ e bla_{IMP}. O gene $bla_{\text{OXA-23}}$ foi detectado em todos os isolados que apresentaram suscetibilidade reduzida aos carbapenêmicos, o que já havia sido relatado por outros autores, mostrando a alta prevalência desta oxacilinase (119,126,169-171).

O bla_{IMP}, que codifica para uma MβL, não foi tão prevalente entre os isolados quanto o $bla_{\text{OXA-23}}$, mas 45,8% dos isolados apresentaram este gene em comparação com o que foi relatado em muitos estudos que se propuseram a investigar a presença desta imipinemase (127,162,166,172-176). Além disso, pôde-se observar, que obtivemos uma boa correlação entre os isolados positivos para o gene bla_{IMP} e o teste de aproximação de discos para detectar a produção de MβL. Entretanto, esta correlação positiva foi possível somente quando analisamos os resultados obtidos com os dois substratos e inibidores conjuntamente, o que nos levou a questionar a precisão, reprodutibilidade e confiabilidade proporcionadas por estes testes, o que já foi levantado por outros autores (129,177-179).

A coexistência de bla_{IMP} e integrons foi detectada em 26,7%, o que poderia ser esperado, uma vez que a maioria dos genes que codifica para imipinemases é encontrada em cassetes inseridos em integrons, especialmente de classe 1 (60,94,98-103,117). Além disto, foi observada a coexistência de bla_{IMP} e $bla_{\text{OXA-23}}$, o que já havia

sido relatado apenas em um trabalho, mas em um número bastante menor de isolados (180).

Além destes dois mecanismos, é possível que os isolados de *A. baumannii* avaliados neste estudo pudessem ter a suscetibilidade reduzida a carbapenêmicos mediada por $bla_{\text{OXA-51}}$, no entanto ainda não podemos afirmar isto porque não foi verificada a presença das sequências de inserção necessárias para o aumento de sua expressão e consequente resistência a este grupo de drogas (137-139).

O tratamento de infecções causadas por CRAB tem sido realizado com polimixinas, polimixina B ou colistina, dependendo da disponibilidade destas drogas em cada país (140,181). Entretanto, já vem sendo relatada a existência de resistência a estes antimicrobianos, mas não na mesma amplitude observada nos carbapenêmicos (145-148). Neste estudo, foram encontrados altos índices de resistência a esta droga, especialmente em isolados não-clínicos, porém mesmo em isolados clínicos foi detectado um percentual maior de resistência comparado a outros estudos. Este dado leva a uma grande preocupação, pois as opções terapêuticas para o tratamento de infecções causadas por *A. baumannii* estão cada vez mais escassas. Porém, drogas pertencentes ao grupo das tetraciclinas, principalmente minociclina e doxiciclina, poderiam ser consideradas para o tratamento de infecções tendo como agentes etiológicos os isolados de *A. baumannii* resistentes à polimixina B avaliados neste trabalho. Tal observação também foi relatada por outros autores em relação a *Acinetobacter* spp. (156,182,183).

Os dados encontrados neste estudo mostram que os isolados apresentaram altas taxas de resistência contra os antimicrobianos testados. Além disso, eles podem carrear genes de resistência que provavelmente estão presentes em elementos genéticos móveis,

tais como integrons, podendo ser transferidos para outros microrganismos. A resistência observada em isolados não-clínicos é especialmente preocupante, pois indica o ambiente da UTI como importante reservatório de *A. baumannii* MDR. A obtenção destes dados também proporcionou ao Serviço de Controle de Infecções do hospital avaliado a implementação de medidas de controle mais adequadas visando contornar os problemas detectados.

Referências Bibliográficas

1. Bergogne-Bérézin E, Towner KJ. *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clinical Microbiology Reviews* 1996; 9:148-65.

2. Towner KJ. *Acinetobacter*: an old friend, but a new enemy. *The Journal of Hospital Infection* 2009; 73:355-63.

3. Koneman EW, Winn WCJr., Allen SD, Janda WM, Procop GW, Schreckenberger PC WG. Diagnóstico microbiológico. texto e atlas colorido. Sexta ed. 2008.

4. Joly-Guillou M-L. Clinical impact and pathogenicity of *Acinetobacter*. *Clinical Microbiology and Infection* 2005; 11:868-73.

5. Obana Y. Pathogenic significance of *Acinetobacter calcoaceticus*: analysis of experimental infection in mice. *Microbiology and Immunology* 1986; 30:645-57.

6. Van Looveren M, Goossens H. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clinical Microbiology and Infection* 2004; 10:684-704.

7. González RH, Dijkshoorn L, Van den Barselaar M, Nudel C. Quorum sensing signal profile of *Acinetobacter* strains from nosocomial and environmental sources. *Revista Argentina de Microbiología* 2009; 41:73-8.

8. Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nature Reviews* 2007; 5:939-51.

9. Gerner-Smidt P, Tjernberg I, Ursing J. Reliability of phenotypic tests for identification of *Acinetobacter* species. *Journal of Clinical Microbiology* 1991; 29:277-82.

10. Henwood CJ, Gatward T, Warner M, James D, Stockdale MW, Spence RP, et al. Antibiotic resistance among clinical isolates of *Acinetobacter* in the UK, and in vitro evaluation of tigecycline (GAR-936). *The Journal of Antimicrobial Chemotherapy* 2002; 49:479-87.

11. Yu Y-S. Typing and characterization of carbapenem-resistant *Acinetobacter calcoaceticus-baumannii* complex in a Chinese hospital. *Journal of Medical Microbiology* 2004; 53:653-656

12. Allen DM HB. *Acinetobacter* species. 6th ed. Philadelphia: Churchill Livingstone: Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 2005.

13. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. *Clinical Mcrobiology Reviews* 2008; 21:538-82.

14. Sunenshine RH, Wright M-O, Maragakis LL, Harris AD, Song X, Hebden J, et al. Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. *Emerging Infectious Diseases* 2007; 13:97-103.

15. Zarrilli R, Casillo R, Di Popolo A, Tripodi M-F, Bagattini M, Cuccurullo S, et al. Molecular epidemiology of a clonal outbreak of multidrug-resistant *Acinetobacter baumannii* in a university hospital in Italy. *Clinical Microbiology and Infection* 2007; 13:481-9.

16. Tsakris A, Ikonomidis A, Poulou A, Spanakis N, Vrizas D, Diomidous M, et al. Clusters of imipenem-resistant *Acinetobacter baumannii* clones producing different carbapenemases in an intensive care unit. *Clinical Microbiology and Infection* 2008; 14:588-94.

17. Centers for disease control and prevention. Healthcare-associated Infections (HAIs) 2010; Available from: http://www.cdc.gov/HAI/organisms/acinetobacter.html

18. World Health Organization. Multidrug-resistant Acinetobacter baumannii (MDRAB) 2010; Available from: http://www.wpro.who.int/media_centre/fact_sheets/fs_20101102.htm

19. Rossi F. The challenges of antimicrobial resistance in Brazil. *Clinical Infectious Diseases* 2011; 52:1138-43.

20. Gales a C, Jones RN, Forward KR, Liñares J, Sader HS, Verhoef J. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997-1999). *Clinical Infectious Diseases* 2001; 32 Suppl 2(Suppl 2):S104-13.

21. Levin AS, Mendes CM, Sinto SI, Sader HS, Scarpitta CR, Rodrigues E, et al. An outbreak of multiresistant *Acinetobacter baumanii* in a university hospital in São Paulo, Brazil. *Infection Control and Hospital Epidemiology* 1996; 17:366-8.

22. Mendes C, Oplustil C, Sakagami E, Turner P, Kiffer C. Antimicrobial susceptibility in intensive care units: MYSTIC Program Brazil 2002. *The Brazilian Journal of Infectious Diseases* 2005; 9:44-51.

23. Fournier PE, Richet H. The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clinical Iinfectious Diseases* 2006; 42:692-9.

24. Paterson DL. The epidemiological profile of infections with multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. *Clinical Infectious Diseases* 2006; 43 Suppl 2(Suppl 2):S43-8.

25. van den Broek PJ, Arends J, Bernards AT, De Brauwer E, Mascini EM, van der Reijden TJK, et al. Epidemiology of multiple *Acinetobacter* outbreaks in The

Netherlands during the period 1999-2001. *Clinical Microbiology and Infection* 2006; 12:837-43.

26. Jawad A, Heritage J, Snelling AM, Gascoyne-Binzi DM, Hawkey PM. Influence of relative humidity and suspending menstrua on survival of *Acinetobacter* spp. on dry surfaces. *Journal of Clinical Microbiology* 1996; 34:2881-7.

27. Hanlon GW. The emergence of multidrug resistant *Acinetobacter* species: a major concern in the hospital setting. *Letters in Applied Microbiology* 2005; 41:375-8.

28. Bernards AT, Frénay HM, Lim BT, Hendriks WD, Dijkshoorn L, van Boven CP. Methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*: an unexpected difference in epidemiologic behavior. *American Journal of Infection Control* 1998; 26:544-51.

29. Bouvet PJM GP. Taxonomy of the Genus *Acinetobacter* with the Recognition of *Acinetobacter baumannii* sp nov., and *Acinetobacter junii* sp nov. and Emended Descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *International Journal of Systematic Bacteriology* 1986; 36:228-240.

30. Ferreira AE, Cunha GR, Fuentefria DP, Corção G. Perfil de suscetibilidade a antimicrobianos em cepas de *Acinetobacter* spp. isoladas de efluente hospitalar em Porto Alegre-RS. Porto Alegre-RS: 2007.

31. Lee N-yao, Chang TC, Wu C-jung, Chang C-ming, Lee H-C, Chen P-L, et al. Clinical manifestations, antimicrobial therapy, and prognostic factors of monomicrobial *Acinetobacter baumannii* complex bacteremia. *Journal of Infection* 2010; 61:219-227.

32. Kaase M, Nordmann P, Wichelhaus T a, Gatermann SG, Bonnin R a, Poirel L. NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. *The Journal of Antimicrobial Chemotherapy* 2011; 66:1260-2.

33. Mendes RE, Bell JM, Turnidge JD, Castanheira M, Jones RN. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. *The Journal of Antimicrobial Chemotherapy* 2009; 63:55-9.

34. Marra AR, Camargo LFA, Pignatari ACC, Sukiennik T, Behar PRP, Medeiros EAS, et al. Nosocomial bloodstream infections in Brazilian Hospitals: Analysis of 2,563 cases from a prospective nationwide surveillance study. *Journal of Clinical Microbiology* 2011; 49:1866-1871.

35. Nemec A, Musílek M, Vaneechoute M, Falsen E, Dijkshoorn L. Lack of evidence for "*Acinetobacter septicus*" as a species different from *Acinetobacter ursingii*? *Journal of Clinical Microbiology* 2008; 46:2826-7;

36. Chang HC, Wei YF, Dijkshoorn L, Vaneechoutte M. Species-Level Identification of Isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* Complex by Sequence Analysis of the 16S-23S rRNA Gene Spacer Region. *Society*. 2005; 43:1632-1639.

37. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant *Acinetobacter baumannii*. *The Journal of Antimicrobial Chemotherapy* 2010; 65:233-8.

38. García-Garmendia JL, Ortiz-Leyba C, Garnacho-Montero J, Jiménez-Jiménez FJ, Pérez-Paredes C, Barrero-Almodóvar AE, et al. Risk factors for *Acinetobacter baumannii* nosocomial bacteremia in critically ill patients: a cohort study. *Clinical Infectious Diseases* 2001; 33:939-46.

39. Bilevicius E, Dragosavac D, Dragosavac S, Araújo S, Falcão AL, Terzi RG. Multiple organ failure in septic patients. *The Brazilian Journal of Infectious Diseases* 2001; 5:103-10.

40. Padoveze MC, Trabasso P, Branchini MLM. Nosocomial infections among HIV-positive and HIV-negative patients in a Brazilian infectious diseases unit. *American Journal of Infection Control* 2002; 30:346-50.

41. Perez F, Hujer AM, Hujer KM, Decker BK, Rather PN, Bonomo R a. Global challenge of multidrug-resistant *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy* 2007; 51:3471-84.

42. Lorenz MG, Wackernagel W. Bacterial gene transfer by natural genetic transformation in the environment. *Microbiological Reviews* 1994; 58:563-602.

43. Metzgar D, Bacher JM, Pezo V, Reader J, Döring V, Schimmel P, et al. *Acinetobacter* sp. ADP1: an ideal model organism for genetic analysis and genome engineering. *Nucleic Acids Research* 2004; 32:5780-90.

44. Gallego L, Towner KJ. Carriage of class 1 integrons and antibiotic resistance in clinical isolates of *Acinetobacter baumannii* from northern Spain. *Journal of Medical Microbiology* 2001; 50:71-7.

45. Gonzalez G, Sossa K, Bello H, Dominguez M, Mella S, Zemelman R. Presence of integrons in isolates of different biotypes of *Acinetobacter baumannii* from Chilean hospitals. *FEMS Microbiology Letters* 1998; 161:125-8.

46. Gu B, Tong M, Zhao W, Liu G, Ning M, Pan S, et al. Prevalence and characterization of class I integrons among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from patients in Nanjing, China. *Journal of Clinical Microbiology* 2007; 45:241-3.

47. Koeleman JG, Stoof J, Van Der Bijl MW, Vandenbroucke-Grauls CM, Savelkoul PH. Identification of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR. *Journal of Clinical Microbiology*; 39:8-13.

48. Kraniotaki E, Manganelli R, Platsouka E, Grossato A, Paniara O, Palù G. Molecular investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii*, with characterisation of class 1 integrons. *International Journal of Antimicrobial Agents* 2006; 28:193-9.

49. Mazel D. Integrons: agents of bacterial evolution. *Nature Reviews* 2006; 4:608-20.

50. Cambray G, Guerout A-M, Mazel D. Integrons. *Annual Review of Genetics* 2010; 44:141-66.

51. BSAC. British Society for Antimicrobial Chemotherapy. 2009;Available from: http://www.bsacsurv.org/

52. Gales a C, Jones RN, Sader HS. Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of Gram-negative bacilli: report from the SENTRY antimicrobial surveillance programme (2001-2004). *Clinical Microbiology and Infection* 2006; 12:315-21.

53. Quinteira S, Sousa JC, Peixe L. Characterization of In100, a new integron carrying a metallo-{beta}-lactamase and a carbenicillinase, from *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy* 2005; 49:451-3.

54. Woodford N, Palepou MF, Babini GS, Holmes B, Livermore DM. Carbapenemases of *Chryseobacterium* (*Flavobacterium*) *meningosepticum*: distribution of blaB and characterization of a novel metallo-beta-lactamase gene, blaB3, in the type strain, NCTC 10016. *Antimicrobial Agents and Chemotherapy* 2000; 44:1448-52.

55. Nicasio AM, Kuti JL, Nicolau DP. The current state of multidrug-resistant gram-negative bacilli in North America. *Pharmacotherapy* 2008; 28:235-49.

56. Breier A MA. Surto de Multirresistência em Porto Alegre – Vigilância Epidemiológica de Casos. XVIII IEA World Congress of Epidemiology. 2008.

57. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clinical Microbiology and Infection* 2006; 12:826-36.

58. Mussi MA, Relling VM, Limansky AS, Viale AM. CarO, an *Acinetobacter baumannii* outer membrane protein involved in carbapenem resistance, is essential for L-ornithine uptake. *FEBS Letters* 2007; 581:5573-8.

59. Siroy A, Molle V, Lemaître-Guillier C, Vallenet D, Pestel-Caron M, Cozzone AJ, et al. Channel formation by CarO, the carbapenem resistance-associated outer membrane protein of *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy* 2005; 49:4876-83.

60. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: the quiet before the storm? *Clinical Microbiology Reviews* 2005; 18:306-25.

61. Brown S, Amyes S. OXA (beta)-lactamases in *Acinetobacter*: the story so far. *The Journal of Antimicrobial Chemotherapy* 2006; 57:1-3.

62. Pournaras S, Markogiannakis a, Ikonomidis a, Kondyli L, Bethimouti K, Maniatis a N, et al. Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in an intensive care unit. *The Journal of Antimicrobial Chemotherapy* 2006; 57:557-61.

63. Martins AF, Kuchenbecker R, Sukiennik T, Boff R, Reiter KC, Lutz L, et al. Carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme: dissemination in Southern Brazil. *Infection* 2009; 37:474-6.

64. Ambler RP. The structure of beta-lactamases. Philosophical transactions of the Royal Society of London. *Series B, Biological Sciences* 1980; 289:321-31.

65. Naas T, Poirel L, Nordmann P. Minor extended-spectrum beta-lactamases. *Clinical Microbiology and Infection*: 2008; 14 Suppl 1:42-52.

66. Poirel L, Villa L, Bertini A, Pitout JD, Nordmann P, Carattoli A. Expanded-spectrum beta-lactamase and plasmid-mediated quinolone resistance. *Emerging Infectious Diseases* 2007; 13:803-5.

67. Poirel L, Mugnier PD, Toleman MA, Walsh TR, Rapoport MJ, Petroni A, et al. ISCR2, another vehicle for *bla*(VEB) gene acquisition. *Antimicrobial Agents and Chemotherapy* 2009; 53:4940-3.

68. Poirel L, Naas T, Guibert M, Chaibi EB, Labia R, Nordmann P. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum beta-lactamase encoded by an *Escherichia coli* integron gene. *Antimicrobial Agents and Chemotherapy* 1999; 43:573-81.

69. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy* 2001; 45:1151-61.

70. Robledo IE, Aquino EE, Santé MI, Santana JL, Otero DM, León CF, et al. Detection of KPC in *Acinetobacter* spp. in Puerto Rico. *Antimicrobial Agents and Chemotherapy* 2010; 54:1354-7.

71. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clinical Microbiology Reviews* 2007; 20:440-58.

72. Jacoby G a. AmpC beta-lactamases. *Clinical Microbiology Reviews* 2009; 22:161-82.

73. Bush K, Jacoby G a. Updated functional classification of beta-lactamases. *Antimicrobial Agents and Chemotherapy* 2010; 54:969-76.

74. Hujer KM, Hamza NS, Hujer AM, Perez F, Helfand MS, Bethel CR, et al. Identification of a new allelic variant of the *Acinetobacter baumannii* cephalosporinase, ADC-7 beta-lactamase: defining a unique family of class C enzymes. *Antimicrobial Agents and Chemotherapy* 2005; 49:2941-8.

75. Nordmann P, Poirel L. Emerging carbapenemases in Gram-negative aerobes. *Clinical Microbiology and infection* 2002; 8:321-31.

76. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? *The Lancet infectious diseases*; 11:381-93.

77. Bush K. Metallo-beta-lactamases: a class apart. *Clinical Infectious Diseases* 1998; 27 Suppl 1:S48-53.

78. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy* 1995; 39:1211-33.

79. Thompson JS, Malamy MH. Sequencing the gene for an imipenem-cefoxitin-hydrolyzing enzyme (CfiA) from *Bacteroides fragilis* TAL2480 reveals strong similarity between CfiA and *Bacillus cereus* beta-lactamase II. *Journal of Bacteriology* 1990; 172:2584-93.

80. Rossolini GM, Franceschini N, Riccio ML, Mercuri PS, Perilli M, Galleni M, et al. Characterization and sequence of the *Chryseobacterium* (*Flavobacterium*) *meningosepticum* carbapenemase: a new molecular class B beta-lactamase showing a broad substrate profile. *The Biochemical Journal* 1998; 332 (Pt 1:145-52.

81. Avison MB, Higgins CS, von Heldreich CJ, Bennett PM, Walsh TR. Plasmid location and molecular heterogeneity of the L1 and L2 beta-lactamase genes of *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy* 2001; 45:413-9.

82. Crowder MW, Walsh TR, Banovic L, Pettit M, Spencer J. Overexpression, purification, and characterization of the cloned metallo-beta-lactamase L1 from *Stenotrophomonas maltophilia*. *Antimicrobial Agents and Chemotherapy* 1998; 42:921-6.

83. Spencer J, Clarke AR, Walsh TR. Novel mechanism of hydrolysis of therapeutic beta-lactams by *Stenotrophomonas maltophilia* L1 metallo-beta-lactamase. *The Journal of Biological Chemistry* 2001; 276:33638-44.

84. Simm AM, Higgins CS, Pullan ST, Avison MB, Niumsup P, Erdozain O, et al. A novel metallo-beta-lactamase, Mbl1b, produced by the environmental bacterium *Caulobacter crescentus*. *FEBS Letters* 2001; 509:350-4.

85. Massidda O, Rossolini GM, Satta G. The *Aeromonas hydrophila* cphA gene: molecular heterogeneity among class B metallo-beta-lactamases. *Journal of Bacteriology* 1991; 173):4611-7.

86. Walsh TR, Gamblin S, Emery DC, MacGowan AP, Bennett PM. Enzyme kinetics and biochemical analysis of ImiS, the metallo-beta-lactamase from *Aeromonas sobria* 163a. *The Journal of Antimicrobial Chemotherapy* 1996; 37:423-31.

87. Walsh TR, Neville WA, Haran MH, Tolson D, Payne DJ, Bateson JH, et al. Nucleotide and amino acid sequences of the metallo-beta-lactamase, ImiS, from *Aeromonas veronii* bv. *sobria*. *Antimicrobial Agents and Chemotherapy* 1998; 42:436-9.

88. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrobial Agents and Chemotherapy*; 44:891-7.

89. Osano E, Arakawa Y, Wacharotayankun R, Ohta M, Horii T, Ito H, et al. Molecular characterization of an enterobacterial metallo beta-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. *Antimicrobial Agents and Chemotherapy* 1994; 38:71-8.

90. Lauretti L, Riccio ML, Mazzariol A, Cornaglia G, Amicosante G, Fontana R, et al. Cloning and characterization of *bla*VIM, a new integron-borne metallo-beta-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrobial Agents and Chemotherapy* 1999; 43:1584-90.

91. Toleman MA, Simm AM, Murphy TA, Gales AC, Biedenbach DJ, Jones RN, et al. Molecular characterization of SPM-1, a novel metallo-beta-lactamase isolated in Latin America: report from the SENTRY antimicrobial surveillance programme. *The Journal of Antimicrobial Chemotherapy* 2002; 50:673-9.

92. Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a beta-lactamase gene, *bla*GIM-1, encoding a new subclass of metallo-beta-lactamase. *Antimicrobial Agents and Chemotherapy* 2004; 48:4654-61.

93. Lee K, Yum JH, Yong D, Lee HM, Kim HD, Docquier J-D, et al. Novel acquired metallo-beta-lactamase gene, *bla*(SIM-1), in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. *Antimicrobial Agents and Chemotherapy* 2005; 49:4485-91.

94. Yong D, Toleman M a, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, *bla*(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrobial Agents and Chemotherapy* 2009; 53:5046-54.

95. Yong D, Bell JM, Ritchie B, Pratt R, Toleman MA WT. A novel sub-group metallo-ß-lactamase (MBL), AIM-1 emerges in *Pseudomonas aeruginosa* (PSA) from Australia. In: 47th Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, USA: 2007. p. C1-1417.

96. Sekiguchi J-ichiro, Morita K, Kitao T, Watanabe N, Okazaki M, Miyoshi-Akiyama T, et al. KHM-1, a novel plasmid-mediated metallo-beta-lactamase from a *Citrobacter freundii* clinical isolate. *Antimicrobial Agents and Chemotherapy* 2008; 52:4194-7.

97. Poirel L, Rodríguez-Martínez J-M, Al Naiemi N, Debets-Ossenkopp YJ, Nordmann P. Characterization of DIM-1, an integron-encoded metallo-beta-lactamase from a *Pseudomonas stutzeri* clinical isolate in the Netherlands. *Antimicrobial Agents and Chemotherapy* 2010; 54:2420-4.

98. Mendes RE, Toleman MA, Ribeiro J, Sader HS, Jones RN, Walsh TR. Integron carrying a novel metallo-beta-lactamase gene, *bla*IMP-16, and a fused form of aminoglycoside-resistant gene aac(6')-30/aac(6')-Ib': report from the SENTRY Antimicrobial Surveillance Program. *Antimicrobial Agents and Chemotherapy* 2004; 48:4693-702.

99. Patzer J, Toleman MA, Deshpande LM, Kamińska W, Dzierzanowska D, Bennett PM, et al. *Pseudomonas aeruginosa* strains harbouring an unusual *bla*VIM-4 gene cassette isolated from hospitalized children in Poland (1998-2001). *The Journal of Antimicrobial Chemotherapy* 2004; 53:451-6.

100. Toleman MA, Biedenbach D, Bennett D, Jones RN, Walsh TR. Genetic characterization of a novel metallo-beta-lactamase gene, *bla*IMP-13, harboured by a novel Tn5051-type transposon disseminating carbapenemase genes in Europe: report from the SENTRY worldwide antimicrobial surveillance programme. *The Journal of Antimicrobial Chemotherapy* 2003; 52:583-90.

101. Walsh TR, Toleman MA, Hryniewicz W, Bennett PM, Jones RN. Evolution of an integron carrying blaVIM-2 in Eastern Europe: report from the SENTRY Antimicrobial Surveillance Program. *The Journal of Antimicrobial Chemotherapy* 2003; 52:116-9.

102. Ramírez MS, Piñeiro S, Centrón D. Novel insights about class 2 integrons from experimental and genomic epidemiology. *Antimicrobial Agents and Chemotherapy* 2010; 54:699-706.

103. Gaur A, Prakash P, Anupurba S, Mohapatra TM. Possible role of integrase gene polymerase chain reaction as an epidemiological marker: study of multidrug-resistant *Acinetobacter baumannii* isolated from nosocomial infections. *International Journal of Antimicrobial Agents* 2007; 29:446-50.

104. Chu YW, Afzal-Shah M, Houang ET, Palepou MI, Lyon DJ, Woodford N, et al. IMP-4, a novel metallo-beta-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. *Antimicrobial Agents and Chemotherapy* 2001; 45:710-4.

105. Da Silva GJ, Correia M, Vital C, Ribeiro G, Sousa JC, Leitão R, et al. Molecular characterization of *bla*(IMP-5), a new integron-borne metallo-beta-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. *FEMS Microbiology Letters* 2002; 215:33-9.

106. Docquier J-D, Riccio ML, Mugnaioli C, Luzzaro F, Endimiani A, Toniolo A, et al. IMP-12, a new plasmid-encoded metallo-beta-lactamase from a *Pseudomonas putida* clinical isolate. *Antimicrobial Agents and Chemotherapy* 2003; 47:1522-8.

107. Iyobe S, Kusadokoro H, Ozaki J, Matsumura N, Minami S, Haruta S, et al. Amino acid substitutions in a variant of IMP-1 metallo-beta-lactamase. *Antimicrobial Agents and Chemotherapy* 2000; 44:2023-7.

108. Iyobe S, Kusadokoro H, Takahashi A, Yomoda S, Okubo T, Nakamura A, et al. Detection of a variant metallo-beta-lactamase, IMP-10, from two unrelated strains of *Pseudomonas aeruginosa* and an *Alcaligenes xylosoxidans* strain. *Antimicrobial Agents and Chemotherapy* 2002; 46:2014-6.

109. Riccio ML, Franceschini N, Boschi L, Caravelli B, Cornaglia G, Fontana R, et al. Characterization of the metallo-beta-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of *bla*(IMP) allelic variants carried by gene cassettes of different phylogeny. *Antimicrobial Agents and Chemotherapy* 2000; 44:1229-35.

110. Yano H, Kuga A, Okamoto R, Kitasato H, Kobayashi T, Inoue M. Plasmid-encoded metallo-beta-lactamase (IMP-6) conferring resistance to carbapenems, especially meropenem. *Antimicrobial Agents and Chemotherapy* 2001; 45:1343-8.

111. Walsh TR. The emergence and implications of metallo-beta-lactamases in Gram-negative bacteria. *Clinical Microbiology and Infection* 2005; 11 Suppl 6:2-9.

112. Bertoncheli, Claudia de Mello RH. Uma revisão sobre metalo-beta-lactamases. *Brazilian Journal of Pharmaceutical Sciences* 2008;44:577-599.

113. Gales AC, Menezes LC, Silbert S, Sader HS. Dissemination in distinct Brazilian regions of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM metallo-beta-lactamase. *The Journal of Antimicrobial Chemotherapy* 2003; 52:699-702.

114. Mendes RE, Castanheira M, Garcia P, Guzman M, Toleman MA, Walsh TR, et al. First isolation of *bla*(VIM-2) in Latin America: report from the SENTRY Antimicrobial Surveillance Program. *Antimicrobial Agents and Chemotherapy*; 48:1433-4.

115. Giamarellou H, Antoniadou A, Kanellakopoulou K. *Acinetobacter baumannii*: a universal threat to public health? *International Journal of Antimicrobial Agents* 2008; 32:106-19.

116. Wang H, Guo P, Sun H, Wang H, Yang Q, Chen M, et al. Molecular epidemiology of clinical isolates of carbapenem-resistant *Acinetobacter* spp. from Chinese hospitals. *Antimicrobial Agents and Chemotherapy* 2007; 51:4022-8.

117. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrobial Agents and Chemotherapy* 2010; 54:24-38.

118. Bou G, Oliver A, Martínez-Beltrán J. OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. *Antimicrobial Agents and Chemotherapy* 2000; 44:1556-61.

119. Villegas MV, Kattan JN, Correa A, Lolans K, Guzman AM, Woodford N, et al. Dissemination of *Acinetobacter baumannii* clones with OXA-23 Carbapenemase in Colombian hospitals. *Antimicrobial Agents and Chemotherapy* 2007; 51:2001-4.

120. Giordano A, Varesi P, Bertini A, Villa L, Dionisi AM, Venditti M, et al. Outbreak of *Acinetobacter baumannii* producing the carbapenem-hydrolyzing oxacillinase OXA-58 in Rome, Italy. *Microbial Drug Resistance* 2007; 13:37-43.

121. Gombac F, Riccio ML, Rossolini GM, Lagatolla C, Tonin E, Monti-Bragadin C, et al. Molecular characterization of integrons in epidemiologically unrelated clinical isolates of *Acinetobacter baumannii* from Italian hospitals reveals a limited diversity of gene cassette arrays. *Antimicrobial Agents and Chemotherapy* 2002; 46:3665-8.

122. Ploy MC, Denis F, Courvalin P, Lambert T. Molecular characterization of integrons in *Acinetobacter baumannii*: description of a hybrid class 2 integron. *Antimicrobial Agents and Chemotherapy* 2000; 44:2684-8.

123. Navia MM, Ruiz J, Vila J. Characterization of an integron carrying a new class D beta-lactamase (OXA-37) in *Acinetobacter baumannii*. *Microbial Drug Resistance* 2002; 8:261-5.

124. Donald HM, Scaife W, Amyes SG, Young HK. Sequence analysis of ARI-1, a novel OXA beta-lactamase, responsible for imipenem resistance in *Acinetobacter baumannii* 6B92. *Antimicrobial Agents and Chemotherapy* 2000; 44:196-9.

125. Naas T, Levy M, Hirschauer C, Marchandin H, Nordmann P. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the carbapenemase OXA-23 in a tertiary care hospital of Papeete, French Polynesia. *Journal of Clinical Microbiology* 2005; 43:4826-9.

126. Dalla-Costa LM, Coelho JM, Souza HAPHM, Castro MES, Stier CJN, Bragagnolo KL, et al. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. *Journal of Clinical Microbiology* 2003; 41:3403-6.

127. Jeong SH, Bae IK, Park KO, An YJ, Sohn SG, Jang SJ, et al. Outbreaks of imipenem-resistant *Acinetobacter baumannii* producing carbapenemases in *Korea. Journal of Microbiology* 2006; 44:423-31.

128. Koh TH, Sng L-H, Wang GCY, Hsu L-Y, Zhao Y. IMP-4 and OXA beta-lactamases in *Acinetobacter baumannii* from Singapore. *The Journal of Antimicrobial Chemotherapy* 2007; 59:627-32.

129. Ferreira AE, Marchetti DP, De Oliveira LM, Gusatti CS, Fuentefria DB, Corção G. Presence of OXA-23-Producing Isolates of *Acinetobacter baumannii* in Wastewater from Hospitals in Southern Brazil. *Microbial Drug Resistance* 2011; 17:221-7.

130. Walther-Rasmussen J, Høiby N. OXA-type carbapenemases. *The Journal of Antimicrobial Chemotherapy* 2006; 57:373-83.

131. Bou G, Cerveró G, Domínguez MA, Quereda C, Martínez-Beltrán J. Characterization of a nosocomial outbreak caused by a multiresistant *Acinetobacter baumannii* strain with a carbapenem-hydrolyzing enzyme: high-level carbapenem resistance in A. baumannii is not due solely to the presence of beta-lactamases. *Journal of Clinical Microbiology* 2000; 38:3299-305.

132. Walsh TR. Emerging carbapenemases: a global perspective. *International Journal of Antimicrobial Agents* 2010; 36 Suppl 3:S8-14.

133. Evans BA, Brown S, Hamouda A, Findlay J, Amyes SGB. Eleven novel OXA-51-like enzymes from clinical isolates of *Acinetobacter baumannii*. *Clinical Microbiology and Infection* 2007; 13:1137-8.

134. Héritier C, Poirel L, Fournier P-edouard, Claverie J-michel, Raoult D, Nordmann P. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy* 2005; 49:4174-9.

135. Brown S, Amyes SGB. The sequences of seven class D beta-lactamases isolated from carbapenem-resistant *Acinetobacter baumannii* from four continents. *Clinical Microbiology and Infection* 2005

136. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the *bla*OXA-51-like carbapenemase gene intrinsic to this species. *Journal of Clinical Microbiology* 2006; 44:2974-6.

137. Figueiredo S, Poirel L, Croize J, Recule C, Nordmann P. In vivo selection of reduced susceptibility to carbapenems in *Acinetobacter baumannii* related to ISAba1-mediated overexpression of the natural *bla*(OXA-66) oxacillinase gene. *Antimicrobial Agents and Chemotherapy* 2009; 53:2657-9.

138. Figueiredo S, Poirel L, Papa A, Koulourida V, Nordmann P. Overexpression of the naturally occurring *bla*OXA-51 gene in *Acinetobacter baumannii* mediated by novel insertion sequence ISAba9. *Antimicrobial Agents and Chemotherapy* 2009; 53:4045-7.

139. Turton JF, Ward ME, Woodford N, Kaufmann ME, Pike R, Livermore DM, et al. The role of ISAba1 in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiology Letters* 2006; 258:72-7.

140. Li J, Rayner CR, Nation RL, Owen RJ, Spelman D, Tan KE, et al. Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy* 2006; 50:2946-50.

141. Reis AO, Luz D a M, Tognim MCB, Sader HS, Gales AC. Polymyxin-resistant *Acinetobacter* spp. isolates: what is next? *Emerging Infectious Diseases* 2003; 9:1025-7.

142. Landman D, Bratu S, Kochar S, Panwar M, Trehan M, Doymaz M, et al. Evolution of antimicrobial resistance among *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* in Brooklyn, NY. *The Journal of Antimicrobial Chemotherapy* 2007; 60:78-82.

143. Landman D, Georgescu C, Martin DA, Quale J. Polymyxins revisited. *Clinical Microbiology Reviews* 2008; 21:449-65.

144. Lim T-P, Tan T-Y, Lee W, Sasikala S, Tan T-T, Hsu L-Y, et al. In-vitro activity of polymyxin B, rifampicin, tigecycline alone and in combination against carbapenem-resistant *Acinetobacter baumannii* in Singapore. *PloS One* 2011; 6(4):e18485.

145. Ko KS, Suh JY, Kwon KT, Jung S-I, Park K-H, Kang CI, et al. High rates of resistance to colistin and polymyxin B in subgroups of *Acinetobacter baumannii* isolates from Korea. *The Journal of Antimicrobial Chemotherapy*; 60:1163-7.

146. Kyoung Park Y. Extreme Drug Resistance in *Acinetobacter baumannii* Infections in Intensive Care Units, South Korea. *Emerging Infectious Diseases* 2009; 15:1325-1327.

147. Park YK, Choi JY, Jung S-I, Park K-H, Lee H, Jung DS, et al. Two distinct clones of carbapenem-resistant *Acinetobacter baumannii* isolates from Korean hospitals. *Diagnostic Microbiology and Infectious Disease* 2009; 64:389-95.

148. Park YK, Lee GH, Baek JY, Chung DR, Peck KR, Song J-H, et al. A single clone of *Acinetobacter baumannii*, ST22, is responsible for high antimicrobial resistance rates of *Acinetobacter* spp. isolates that cause bacteremia and urinary tract infections in Korea. *Microbial Drug Resistance* 2010; 16:143-9.

149. Dizbay M, Tozlu DK, Cirak MY, Isik Y, Ozdemir K, Arman D. In vitro synergistic activity of tigecycline and colistin against XDR-*Acinetobacter baumannii*. *The Journal of Antibiotics* 2010; 63:51-3.

150. Schafer JJ, Goff DA, Stevenson KB, Mangino JE. Early experience with tigecycline for ventilator-associated pneumonia and bacteremia caused by multidrug-resistant *Acinetobacter baumannii*. *Pharmacotherapy* 2007; 27:980-7.

151. Pachón-Ibáñez ME, Jiménez-Mejías ME, Pichardo C, Llanos AC, Pachón J. Activity of tigecycline (GAR-936) against *Acinetobacter baumannii* strains, including those resistant to imipenem. *Antimicrobial Agents and Chemotherapy* 2004; 48:4479-81.

152. Navon-Venezia S, Leavitt A, Carmeli Y. High tigecycline resistance in multidrug-resistant *Acinetobacter baumannii*. *The Journal of Antimicrobial Chemotherapy* 2007; 59:772-4.

153. Golanbar GD, Lam CK, Chu Y-M, Cueva C, Tan SW, Silva I, et al. Phenotypic and molecular characterization of *Acinetobacter* clinical isolates obtained from inmates of california correctional facilities. *Journal of Clinical Microbiology* 2011; 49:2121-31.

154. Young LS, Sabel AL, Price CS. Epidemiologic, clinical, and economic evaluation of an outbreak of clonal multidrug-resistant *Acinetobacter baumannii* infection in a surgical intensive care unit. *Infection Control and Hospital Epidemiology* 2007; 28:1247-54.

155. Valenzuela JK, Thomas L, Partridge SR, van der Reijden T, Dijkshoorn L, Iredell J. Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. *Journal of Clinical Microbiology* 2007; 45:453-60.

156. Valentine SC, Contreras D, Tan S, Real LJ, Chu S, Xu HH. Phenotypic and molecular characterization of *Acinetobacter baumannii* clinical isolates from nosocomial outbreaks in Los Angeles County, California. *Journal of Clinical Microbiology* 2008; 46:2499-507.

157. Donnarumma F, Sergi S, Indorato C, Mastromei G, Monnanni R, Nicoletti P, et al. Molecular characterization of *Acinetobacter* isolates collected in intensive care units of six hospitals in Florence, Italy, during a 3-year surveillance program: a population structure analysis. *Journal of Clinical Microbiology* 2010; 48:1297-304.

158. Chen T-L, Lee Y-T, Kuo S-C, Hsueh P-R, Chang F-Y, Siu L-K, et al. Emergence and Distribution of Plasmids Bearing the blaOXA-51-like gene with an upstream ISAba1 in carbapenem-resistant *Acinetobacter baumannii* isolates in Taiwan. *Antimicrobial Agents and Chemotherapy* 2010; 54:4575-81.

159. Lee Y-T, Turton JF, Chen T-L, Wu RC-C, Chang W-C, Fung C-P, et al. First identification of blaOXA-51-like in non-*baumannii Acinetobacter* spp. *Journal of Chemotherapy* 2009; 21:514-20.

160. Fontana C, Favaro M, Minelli S, Bossa MC, Testore GP, Leonardis F, et al. *Acinetobacter baumannii* in intensive care unit: a novel system to study clonal relationship among the isolates. *BMC Infectious Diseases* 2008; 8:79.

161. Wilson APR, Smyth D, Moore G, Singleton J, Jackson R, Gant V, et al. The impact of enhanced cleaning within the intensive care unit on contamination of the near-patient environment with hospital pathogens: a randomized crossover

study in critical care units in two hospitals. *Critical Care Medicine* 2011; 39:651-8.

162. Touati A, Achour W, Cherif A, Hmida HB, Afif FB, Jabnoun S, et al. Outbreak of *Acinetobacter baumannii* in a neonatal intensive care unit: antimicrobial susceptibility and genotyping analysis. *Annals of Epidemiology* 2009; 19:372-8.

163. Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. *European Journal of Clinical Microbiology & Infectious Diseases* 1999; 18:761-70.

164. Rowe-Magnus DA, Mazel D. Resistance gene capture. *Current Opinion in Microbiology* 1999; 2:483-8.

165. Yan Z-Q, Shen D-X, Cao J-R, Chen R, Wei X, Liu L-P, et al. Susceptibility patterns and molecular epidemiology of multidrug-resistant *Acinetobacter baumannii* strains from three military hospitals in China. *International Journal of Antimicrobial Agents* 2010; 35:269-73.

166. Lin M-F, Chang K-C, Lan C-Y, Chou J, Kuo J-W, Chang C-K, et al. Molecular epidemiology and antimicrobial resistance determinants of multidrug-resistant *Acinetobacter baumannii* in five proximal hospitals in Taiwan. *Japanese Journal of Infectious Diseases* 2011; 64:222-7.

167. Turton JF, Kaufmann ME, Glover J, Coelho JM, Warner M, Pike R, et al. Detection and typing of integrons in epidemic strains of *Acinetobacter baumannii* found in the United Kingdom. *Journal of Clinical Microbiology* 2005; 43:3074-82.

168. Kansakar P, Dorji D, Chongtrakool P, Mingmongkolchai S, Mokmake B, Dubbs P. Local dissemination of multidrug-resistant *Acinetobacter baumannii* clones in a Thai hospital. *Microbial Drug Resistance* 2011; 17:109-19.

169. Niumsup PR, Boonkerd N, Tansawai U, Tiloklurs M. Carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 in Thailand. *Japanese Journal of Infectious Diseases* 2009; 62:152-4.

170. Zhou H, Pi B-R, Yang Q, Yu Y-S, Chen Y-G, Li L-J, et al. Dissemination of imipenem-resistant *Acinetobacter baumannii* strains carrying the ISAba1 *bla*OXA-23 genes in a Chinese hospital. *Journal of Medical Microbiology* 2007; 56:1076-80.

171. Stoeva T, Higgins PG, Savov E, Markovska R, Mitov I, Seifert H. Nosocomial spread of OXA-23 and OXA-58 beta-lactamase-producing *Acinetobacter baumannii* in a Bulgarian hospital. *The Journal of Antimicrobial Chemotherapy* 2009; 63:618-20.

172. Koo SH, Kwon KC, Cho HH, Sung JY. Genetic basis of multidrug-resistant *Acinetobacter baumannii* clinical isolates from three university hospitals in

Chungcheong Province, Korea. *The Korean Journal of Laboratory Medicine* 2010; 30:498-506.

173. Lee M-F, Peng C-F, Hsu H-J, Chen Y-H. Molecular characterisation of the metallo-beta-lactamase genes in imipenem-resistant Gram-negative bacteria from a university hospital in southern Taiwan. *International Journal of Antimicrobial Agents* 2008; 32:475-80.

174. Carvalho KR, Carvalho-Assef APD, Peirano G, Santos LCGD, Pereira MJF, Asensi MD. Dissemination of multidrug-resistant *Acinetobacter baumannii* genotypes carrying *bla*(OXA-23) collected from hospitals in Rio de Janeiro, Brazil. *International Journal of Antimicrobial Agents* 2009; 34:25-8.

175. Lin W-R, Lu P-L, Siu L-K, Chen T-C, Lin C-Y, Hung C-T, et al. Rapid control of a hospital-wide outbreak caused by extensively drug-resistant OXA-72-producing *Acinetobacter baumannii*. *The Kaohsiung Journal of Medical Sciences* 2011; 27:207-14.

176. Kulah C, Mooij MJ, Comert F, Aktas E, Celebi G, Ozlu N, et al. Characterisation of carbapenem-resistant *Acinetobacter baumannii* outbreak strains producing OXA-58 in Turkey. *International Journal of Antimicrobial Agents* 2010; 36:114-8.

177. Uma Karthika R, Srinivasa Rao R, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, et al. Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of *Acinetobacter baumannii* from a South Indian tertiary care hospital. *Journal of Medical Microbiology* 2009; 58: 430-5.

178. Gupta V, Datta P, Chander J. Prevalence of metallo-beta lactamase (MBL) producing *Pseudomonas* spp. and *Acinetobacter* spp. in a tertiary care hospital in India. *The Journal of Infection* 2006; 52:311-4.

179. Noyal MJC, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. *The Indian Journal of Medical Research* 2009; 129:707-12.

180. Koh TH, Tan TT, Khoo CT, Ng SY, Tan TY, Hsu L-Y, et al. *Acinetobacter calcoaceticus*- *Acinetobacter baumannii* complex species in clinical specimens in Singapore. *Epidemiology and Infection* 2011: 1-4.

181. Kallel H, Hergafi L, Bahloul M, Hakim A, Dammak H, Chelly H, et al. Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: a matched case-control study. *Intensive Care Medicine* 2007; 33:1162-7.

182. Draghi DC, Tench S, Dowzicky MJ, Sahm DF. Baseline in vitro activity of tigecycline among key bacterial pathogens exhibiting multidrug resistance. *Chemotherapy* 2008; 54:91-100.

183. Akers KS, Mende K, Yun HC, Hospenthal DR, Beckius ML, Yu X, et al. Tetracycline susceptibility testing and resistance genes in isolates of *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex from a U.S. military hospital. *Antimicrobial Agents and Chemotherapy* 2009; 53:2693-5.

Anexo 1

Guia para autores

Journal of Infection

Official Journal of the British Infection Association

Guide for Authors

The Journal of Infection publishes original papers on all aspects of infection - clinical, microbiological and epidemiological. The Journal seeks to bring together knowledge from all specialties involved in infection research and clinical practice, and present the best work in the ever-changing field of infection.

Each issue brings you Editorials that describe current or controversial topics of interest, high quality Reviews to keep you in touch with the latest developments in specific fields of interest, an Epidemiology section reporting studies in the hospital and the general community, and a lively correspondence section.

The Journal of Infection will consider for publication an original article, review, case report or letter to the Editor on any aspect of infection written concisely in English provided it is not being considered for publication elsewhere.

This journal does not encourage resubmission of rejected papers.

Authors must comply fully with these instructions. These guidelines generally follow the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" The complete document appears at http://www.icmje.org.

Online Submission

Manuscripts by online submission only. Submit your manuscript at http://ees.elsevier.com/yjinf/. Use the following guidelines to prepare your article. Visit (http://www.elsevier.com/authors) you will be guided stepwise through the creation and uploading of the various files. The system automatically converts source files to a single Adobe Acrobat PDF version of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail and via the Author's homepage, removing

the need for a hard-copy paper trail. The above represents a very brief outline of this form of submission. It can be advantageous to print this "Guide for Authors" section from the site for reference in the subsequent stages of article preparation.

Online-only publication

In addition to publishing case reports online-only, Journal of Infection is now offering this option to original scientific work. The authors of papers accepted for publication may be offered either (i) print and online publication or (ii) online-only publication. Offers will be made at the discretion of the Editor. For further details please refer to the editorial published in the journal (J Infect 2006; 53: 47).

Ethics

Work on human beings that is submitted to Journal of Infection should comply with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines.

Studies on patients or volunteers require ethics committee approval and informed consent which should be documented in your paper. Patients have a right to privacy. Therefore identifying information, including patients' images, names, initials, or hospital numbers, should not be included in videos, recordings, written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and you have obtained written informed consent for publication in print and electronic form from the patient (or parent, guardian or next of kin where applicable). If such consent is made subject to any conditions, Elsevier must be made aware of all such conditions. Written consents must be provided to Elsevier on request. Even where consent has been given, identifying details should be omitted if they are not essential. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning and editors should so note. If such consent has not been obtained, personal details of patients included in any part of the paper and in any supplementary materials (including all illustrations and videos) must be removed before submission.

Randomised controlled trials

All randomised controlled trials submitted for publication in Journal of Infection should include a completed Consolidated Standards of Reporting Trials (CONSORT) flow

chart. Please refer to the CONSORT statement website at http://www.consort-statement.org for more information. Journal of Infection has adopted the proposal from the International Committee of Medical Journal Editors (ICMJE) which require, as a condition of consideration for publication of clinical trials, registration in a public trials registry. Trials must register at or before the onset of patient enrolment. The clinical trial registration number should be included at the end of the abstract of the article. For this purpose, a clinical trial is defined as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g. phase I trials) would be exempt. Further information can be found at http://www.icmje.org.

Digital Object Identifier

The digital object identifier (DOI) may be used to cite and link to electronic documents. The DOI consists of a unique alphanumeric character string, which is assigned to a document by the publisher upon the initial electronic publication. The assigned DOI never changes. Therefore, it is an ideal medium for citing a document, particularly 'Articles in press' because they have not yet received their full bibliographic information. The correct format for citing a DOI is shown as follows (Example taken from a document in the journal): doi:10.1016/j.inf.2004.05.019 when you use the DOI to create URL hyperlinks to documents on the web, they are guaranteed never to change.

Language Editing

International Science Editing and Asia Science Editing can provide English language and copyediting services to authors who want to publish in scientific, technical and medical journals, and need assistance before they submit their article of before it is accepted for publication. Authors can contact these services directly: International Science Editing (http://www.internationalscienceediting.com) and Asia Science Editing (http://www.asiascienceediting.com) or, for more information about language editing services, please contact authorsupport@elsevier.com who will be happy to deal with any questions. Please note Elsevier neither endorses nor takes responsibility for any products, goods or services offered by outside vendors through our services or in any advertising. For more information please refer to our terms and conditions (http://www.elsevier.com/authors).

Title

Your title page, numbered as 1, should give the title in capital letters (not exceeding 100 letters), a running title (not exceeding 50 letters) and the authors' names (as they are to appear), affiliations and complete addresses, including postal (zip) codes. The author and address to whom correspondence should be sent must be clearly indicated. Please supply telephone, fax and e-mail numbers for the corresponding author. All authors should have made substantial contributions to all of the following: (1) the conception

and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted.

Abstracts

A structured abstract of your manuscript, a maximum of 200 words, summarizing the content, should be provided on a separate sheet following the title page. This must be divided into sections entitled Objectives; Methods; Results; Conclusions.

Length

The text should comprise the following sections: Summary, Key words, Introduction, Materials (or Patients) and Methods, Results, Discussion, Acknowledgements and References. Tables, Figures and Legends to figures should be on separate sheets.

Reference Format

References must accord with the 'Vancouver' style (Br Med J 1982; 284: 1766-1770). When the number of authors is six or less, the names and initials of all should be given in the reference list; when seven or more, the first six names should be cited, followed by et al. Authors are responsible for checking the accuracy of all references and ensuring that all given in the text agree with those in the reference list. Example: 1. McConkey B, Crockson R A, Crockson A P, Wilkinson A R. The effect of some anti-inflammatory drugs on the acute phase proteins in rheumatoid arthritis. Q J Med 1973; 42: 785-791. References to book chapters should be set out: Example: 1. Weinstein L, Schwartz M N. Pathogenic properties of invading micro-organisms. In: Soderman W A Jr, Soderman W A, eds. Pathologic physiology: mechanisms of disease. W B Saunders, 1974: 457-472. References cited solely in tables and/or legends to figures should be numbered in accordance with the sequence established by the first mention in the text of the table or illustration. Papers submitted with references or other features which fail to comply with these instructions will be returned, and not considered for publication until resubmitted in the required style. When citing an Elsevier journal, include the digital object identifier (DOI), if noted, from the article's title page. Please note the following examples. 1. Munday PE. Pelvic inflammatory disease - an evidence-based approach to diagnosis. J Infect 2000; 40: 31-41, doi:10.1053/jinf.1999.0609 2. Colebunders R, Borchert M. Ebola haemorrhagic fever - a review. J Infect, doi: 10.1053/jinf. 1999.0603.

DNA sequences and GenBank accession numbers

Many journals cite "gene accession numbers" in their running text and footnotes. Gene accession numbers refer to genes or DNA sequences about which further information can be found in the databases at the National Center for Biotechnical Information (NCBI) at the National Library of Medicine. Elsevier authors wishing to enable other scientists to use the accession numbers cited in their papers via links to these sources, should type this information in the following manner:For each and every accession

number cited in an article, authors should type the accession number in bold, underlined text. Letters in the accession number should always be capitalised (see example below). This combitnation of letters and format will enable Elsevier's typesetters to recognise the relevant texts as accession numbers and add the required link to GenBank's sequences. Example: "GenBank accession nos. AI631510 , AI631511 , AI632198 , and BF223228), a B-cell tumor from a chronic lymphatic leukemia (Gen Bank accession no. BE675048), and a T-cell lymphoma (GenBank accession no. AA361117)". Authors are encouraged to check accession numbers used very carefully. An error in a letter or number can result in a dead link. In the final version of the printed article , the accession number text will not appear bold or underlined. In the final version of the electronic copy , the accession number text will be linked to the appropriate source in the NCBI databases enabling readers to go directly to that source from the article.

Figures

Illustrations should be numbered in Arabic numerals (e.g. Fig. 3). Each figure should have a title that makes its meaning clear without reference to the text. For graphs and illustrations, high resolution files (jpeg or tiff) should be submitted. For each illustration a brief legend should be typewritten on a separate sheet. The identity of patients shown in photographs should either be concealed, or they should give written consent; a copy of consent should accompany the photograph. Simple masking of eyes in a photograph may not be sufficient. Colour prints will be considered only if essential; reproduction is at the author's expense. For colour reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article. For further information on the preparation of electronic artwork, please see artwork instructions

Tables

Tables should be numbered in Arabic numerals (e.g. Table 3). Each one should be on a separate sheet and have a title that makes its meaning clear without reference to the text included.

Acknowledgements

All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

Role of the funding source

All sources of funding should be declared as an acknowledgement at the end of the text. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

Preparation of supplementary data

Elsevier now accepts electronic supplementary material to support and enhance your scientific research. Supplementary files offer the author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier web products, including ScienceDirect: http://www.elsevier.com/authors. In order to ensure that your submitted material is directly usable, please ensure that data is provided in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file.

Keywords

Authors should provide Keywords from their summary. They should include those published in Medical Subject Headings - Annotated Alphabetical List, U.S. Department of Health and Human Services (new volume every year).

Units and Abbreviations

Microbes should be referred to by their scientific names according to the binominal system used in the latest edition of Bergey's Manual of Systematic Bacteriology (The Williams and Wilkins Co.). When first mentioned, the name should be in full and underlined - to denote italics. Thenceforward, the genus should be abbreviated to its initial letter, e.g. 'S. aureus' not 'Staph. aureus'. If abbreviation is likely to cause confusion or render the intended meaning(s) unclear the names of organisms should be given in full. Only those names included in the Approved Lists of Bacterial Names (Int J Syst Bacteriol 1980; 30: 225-420) and/or which have been validly published in the Int J Syst Bacteriol since January 1980 are acceptable. If there is a good reason to use a name that does not have standing in nomenclature, it should be enclosed in quotation marks and an appropriate statement concerning its use made in the text (e.g. Int J Syst Bacteriol 1980; 30: 547-556). Symbols for units of measurement must accord with the Systeme International (SI). However, blood pressure should be expressed in mmHg and haemoglobin as g/dl. Drugs should be referred to by their generic, and not proprietary, name(s); for guidance the latest edition of the British National Formulary should be consulted.

Case Reports

Case reports should not exceed 2000 words, with no more than one table and figure included. Case reports do not require a structured abstract. Please note that a high number of case reports are submitted to the journal and only a small percentage can reasonably be accepted for publication. Case reports are now published online only.

Copyright Information

Upon acceptance of an article, authors will be asked to sign a "Journal Publishing Agreement" (for more information on this and copyright see http://www.elsevier.com/authors). Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail (or letter) will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form. Authors are responsible for obtaining from the copyright holder permission to reproduce any figures for which copyright exists.

Proofs

One set of page proofs in PDF format will be sent by e-mail to the corresponding Author (if we do not have an e-mail address then paper proofs will be sent by post). Elsevier now sends PDF proofs which can be annotated; for this you will need to download Adobe Reader version 7 available free from http://www.adobe.com/products/acrobat/readstep2.html. Instructions on how to annotate PDF files will accompany the proofs. The exact system requirements are given at the Adobe site: http://www.adobe.com/products/acrobat/acrrsystemreqs.html#70win If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post. Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. Therefore, it is important to ensure that all of your corrections are sent back to us in one communication: please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Electronic offprints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail or, alternatively, 25 free paper offprints. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use. Additional paper offprints can be ordered by the authors. An order form with prices will be sent to the corresponding author.

Author Enquiries

For enquiries relating to the submission of articles (including electronic submission where available) please visit http://www.elsevier.com/authors. This also provides information to track accepted articles and set up e-mail alerts to inform you of when an article's status has changed, as well as detailed artwork guidelines, copyright

information, frequently asked questions and more. Contact details for questions arising after acceptance of an article, especially those relating to proofs, are provided after registration of an article for publication.

Funding body agreements and policies

Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies

Disclaimer

The opinions expressed in the Journal are those of the authors, and not necessarily the Editorial Board or publishers. Ultimate responsibility in use and checking drug doses mentioned in the Journal, an in interpretation of published material lies with the physician concerned. Neither the Editorial Board nor the publishers can accept any liability whatsoever in respect of a claim for damages arising there from.