UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Instituto de Ciências Biomédicas
Programa de Pós-Graduação em Imunologia e Parasitologia Aplicadas

Epidemiologia de Infecções Relacionadas à Assistência à Saúde causadas por bacilos Gram-negativos em Uberlândia - MG

Vinícius Lopes Dias

Uberlândia - MG
2023

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Tese apresentada ao Colegiado do Programa de Pós-graduação em Imunologia e Parasitologia Aplicadas como requisito parcial para obtenção do título de Doutor.

Vinícius Lopes Dias
Orientadora: Profa. Dra. Rosineide Marques Ribas
Coorientador: Prof. Dr. Paulo Pinto Gontijo-Filho

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UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Coordenação do Programa de Pós-Graduação em Imunologia e Parasitologia Aplicada
Av. Amazonas, s/n, Bloco 4C, Sala 4C218 - Bairro Umuarama, Uberlândia-MG, CEP 38400-902
Telefone: (34) 3225-8672 - www.imunoparasito.ufu.br - coipa@ufu.br

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No dia 01 de agosto de 2023, reuniu-se, por vídeo conferência, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Imunologia e Parasitologia Aplicadas, assim composta pelo Presidente(a): Rosineide Marques Ribas - Universidade Federal de Uberlândia /UFU; e os Titulares: Helisângela de Almeida Silva - Universidade Federal de Uberlândia /UFU; Paola Amaral de Campos - Universidade Federal de Uberlândia /UFU; Dayane Otero Rodrigues - Universidade Federal do Oeste da Bahia/UFOB; Michel Rodrigues Moreira - Universidade Federal de Juiz de Fora/UFJF.

Iniciando os trabalhos a presidente da mesa, Profa. Rosineide Marques Ribas, apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente, o tempo de arguição e resposta foram conforme as normas do Programa.

A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

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Dedico este trabalho com muito amor e gratidão aos que sempre me apoiaram nesta trajetória

“For me, success is a state of mind. I feel like success isn’t about conquering something; it’s being happy with who you are.”

Britney Spears

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RESUMO

A preocupação com a rápida emergência e disseminação de microrganismos resistentes é crescente no Brasil e no mundo, uma vez que as opções terapêuticas clinicamente disponíveis para o tratamento de infecções causadas por estes são limitadas, resultando em altas taxas de mortalidade. A epidemiologia e o controle das infecções causadas por bactérias resistentes podem ser favorecidos pela análise epidemiológica, bem como a análise molecular dessas cepas. Dessa forma, este trabalho buscou elementos para compreender a epidemiologia de infecções relacionadas à assistência à saúde (IRAS) causadas por bacilos Gram-negativos (BGN) nos últimos 10 anos, incluindo a análise de genes de resistência, virulência e disseminação de cepas de *Klebsiella pneumoniae* resistentes aos carbapenêmicos na cidade de Uberlândia. Os dados evidenciaram um aumento na incidência de IRAS na última década, bem como uma alta taxa (67,4%) de resistência aos carbapenêmicos nos principais três espécies de bacilos Gram-negativos (*Acinetobacter baumannii, K. pneumoniae* e *Pseudomonas aeruginosa*) causadores de IRAS. Dados coletados também revelaram que as taxas de infecções causadas por estes três microrganismos aumentaram em aproximadamente seis vezes entre os anos de 2011 e 2018. Em relação a presença dos genes de resistência e virulência em cepas de *K. pneumoniae*, observou-se que, 47,9% (79), 10,9% (18), e 72,7% (120) carreavam os genes bla_{KPC}, bla_{NDM}, e bla_{VIM} respectivamente, enquanto 91,8% carreavam simultaneamente os genes *entb*, *kfu*, *ureA*, and *wabG*. Quanto a análise clonal de um grupo de 15 isolados selecionados dessas 165 amostras, a análise de similaridade detectou a presença de cinco padrões clonais (A-E), sendo o pulsotipo B o mais prevalente, favorecendo compreensão da dinâmica da disseminação dessa cepa resistente no ambiente hospitalar.

Os resultados desses estudos evidenciam uma frequência alarmante de microrganismos como *A. baumannii* (41,5%), *P. aeruginosa* (29,9%) e *K. pneumoniae* (41,5%) resistente aos carbapenêmicos, representando uma ameaça significativa para os serviços de saúde, uma vez que esta resistência está relacionada a um mau prognóstico. As IRAS causadas por microrganismos multirresistentes, principalmente *K. pneumoniae* representam um desafio em termos de controle de infecção e tratamento para pacientes internados e não internados em UTI, conforme evidenciado pelos achados deste estudo. As características de transmissão e os mecanismos de resistência e virulência associados a esse patógeno merecem maior atenção e investigação.

Palavras-chave: Resistência a Múltiplos Medicamentos, IRAS, Virulência, *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa,* Clonalidade.

ABSTRACT

The concern about the rapid emergence and spread of resistant microorganisms is growing in Brazil and worldwide, as the clinically available therapeutic options for treating infections caused by these microorganisms are limited, resulting in high mortality rates. The epidemiology and control of infections caused by resistant bacteria can be facilitated by epidemiological analysis as well as molecular analysis of these strains. Thus, this study sought elements to understand the epidemiology of healthcare-associated infections (HAIs) caused by Gram-negative bacilli (GNB) in the last 10 years, including the analysis of resistance, virulence, and dissemination genes of carbapenem-resistant *K. pneumoniae* strains in the city of Uberlândia. The data evidenced an increase in the incidence of HAIs in the last decade, as well as a high rate (67.4%) of carbapenem resistance in the three main species of Gram-negative bacilli (*Acinetobacter baumannii, K. pneumoniae*, and *Pseudomonas aeruginosa*) causing HAIs. Collected data also revealed that the infection rates for these three microorganisms increased approximately sixfold between 2011 and 2018. Regarding the presence of resistance and virulence genes in *K. pneumoniae* strains, it was observed that 47.9% (79), 10.9% (18) and 72.7% (120) carried the bla_{KPC}, bla_{NDM}, and bla_{VIM} genes, respectively, while 91.8% simultaneously carried the *entb*, *kfu*, *ureA*, and *wabG* genes. As for the clonal analysis of a group of 15 selected isolates from these 165 samples, the similarity analysis detected the presence of five clonal patterns (A-E), with pulsotype B being the most prevalent, facilitating the understanding of the dynamics of dissemination of this resistant strain in the hospital environment. The results of this study highlight an alarming frequency of microorganisms such as *A. baumannii* (41.5%), *P. aeruginosa* (29.9%), and carbapenem-resistant *K. pneumoniae* (28.6%), representing a significant threat to healthcare services, as this resistance is associated with a poor prognosis. HAIs caused by multidrug-resistant microorganisms, particularly *K. pneumoniae*, represent a significant challenge in terms of infection control and treatment for both hospitalized and non-hospitalized patients in the ICU, as evidenced by the findings of this study. The transmission characteristics and the resistance and virulence mechanisms associated with this pathogen deserve more attention and investigation.

Keywords: Multidrug Resistance, HAI, Virulence, *Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa*, Clonality.

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

>	Maior
≥	Maior ou igual
≤	Menor ou igual
%	Porcentagem
°C	Graus Celsius
µL	Microlitros
β	Beta
AIDS	*Acquired Immuno-Deficiency Syndrome;* Síndrome da Imunodeficiência Adquirida
AIM-1	*Australian Imipenemase;* Imipinase Australiana
BKC-1	*Brazilian Klebsiella carbapenemase-1; Klebsiella* carbapenemase-1 brasileira
BGN	Bacilos Gram-negativos
BHI	*Brain Heart Infusion; Caldo* infusão cérebro-coração
BSI	*Bloodstream infections*; Infecções de corrente sanguínea
CDC	*Centers for Disease Control and Prevention;* Centros de Controle e Prevenção de Doenças
CI	*Confidence interval;* Intervalo de confidência
CLSI	*Clinical and Laboratory Standard Institute*; Instituto de Padrões Clínicos e Laboratoriais
CRAB	*Carbapenem-resistant Acinetobacter baumannii; A. baumannii* resistente aos carbapenêmicos
CRE	*Carbapenem-resistant Enterobacteriaceae*; Enterobacteriaceae resistente aos carbapenêmicos
CRKP	Carbapenem-resistant *K. pneumoniae; K. pneumoniae* resistente aos carbapenêmicos
CVC	Cateter venoso central
DIM-1	*Dutch imipenemase;* Imipenemase holandesa
DNA	*Desoxyribonucleic Acid*; Ácido Desoxirribonucleico
EDTA	*Ethylenediamine tetraacetic acid*; Ácido etileno-diamino tetracético
ESBL	*Extended-spectrum β-lactamase*; β-lactamases de amplo espectro

et al.	E colaboradores
GIM	*German Imipenemase;* Imipenemase alemã
GNB	*Gram-negative bacilli*; Bacilos Gram-negativos
h	Horas
HAI	*Healthcare associated infection;* Infecções relacionadas à assistência à saúde
IRAS	Infecções relacionadas à assistência à saúde
IMP	*Imipenemase*
ICU	*Intense Care Unit;* Unidade de Terapia Intensiva
KHM	*Kyorin University Hospital;* Hospital Universitário de Kyorin
KPC-2	*KPC-2-producing Klebsiella pneumoniae*; *Klebsiella pneumoniae* produtoras de carbapenemases do tipo KPC-2
LPS	Lipopolissacarídeo
MBL	Metalo-β-lactamases
MDR	*Multidrug-Resistant*; Multirresistente
MDRGN	*Multidrug-resistant Gram-negative bactéria;* Bacilos Gram-negativos multirressistentes
mg	Miligrama
mL	Mililitro
mm	Milímetro
n	*Number;* Número
NDM-1	*New Delhi MBL;* MBL de Nova Delhi
nm	Nanômetro
NMDR	*non – Multidrug Resistant;* Não MDR
OMS	Organização Mundial de Saúde
OR	*Odds ratio;* Razão de probabilidades
OXA	Oxacilinases
p	Valor de p
PCR	*Polymerase Chain Reaction*; Reação em cadeia da polimerase
PFGE	*Pulsed-field gel electrophoresis*; Eletroforese em gel de campo pulsado
SENTRY	*Antimicrobial Surveillance Program*; Programa de vigilância antimicrobiana
SIM-1	*Seul Imipenemase;* Imipenemase de Seul

SPM	*São Paulo MBL;* MBL de São Paulo
ST	*Sequence type;* Tipagem de sequência
TBE	*Tris, Borate and EDTA*; Tris, Ácido bórico e EDTA
TE	Tris/EDTA
TSB	*Tryptic soy broth*; Caldo Triptona Soja
UPGMA	*Unweighted Pair Group Method Using Arithmetic Averages*; Método de grupo de pares não ponderados usando médias aritméticas
UTI	Unidade de Terapia Intensiva
VIM	*Verona Imipenemase;* Imipenemase de Verona
VRE	*Vancomycin-resistant Enterococcus faecium; E. faecium* resistente à vancomicina
WHO	*World Health Organization;* Organização Mundial da Saúde

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SUMÁRIO

Apresentação

Ao longo dos anos, a alta incidência de microrganismos multirresistentes clinicamente importantes tornou-se um problema global de saúde, especialmente em países de baixa e média renda como o Brasil, sendo um fenômeno cosmopolita endêmico. Nesse sentido, diferentes espécies com destaque para *Acinetobacter baumannii*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* e *Staphylococcus aureus* adquiriram e/ou desenvolveram mecanismos de resistência aos antimicrobianos de última geração, comprometendo de forma significativa o tratamento levando ao um impacto negativo no prognóstico dos pacientes.

Infelizmente o problema da resistência aos antimicrobianos tornou-se ainda mais preocupante com o fato de que não estar mais restrito aos ambientes hospitalares. De acordo com a literatura mundial, existe a preocupação explícita de que ambientes aquáticos, alimentos e animais de produção que tenham sido diretas ou indiretamente expostos a antimicrobianos, contribuído para o estabelecimento de bactérias resistentes em sua microbiota.

Muitos estudos tem demonstrado que a emergência, sobrevivência e adaptabilidade bacteriana em diferentes ambientes tem sido facilitada pela aquisição de genes e/ou elementos móveis principalmente através da recombinação gênica e/ou mutações. Vale ressaltar que atualmente essa disseminação de resistência entre os diferentes ecossistemas e os hospedeiros tem sido discutida dentro de um conceito "*one-health*" que tem ganhado mais notoriedade recentemente e que pode assegurar a aplicação de boas práticas relacionadas a prevenção, controle, vigilância e detecção dessas infecções.

Os mecanismos de resistência de bacilos Gram-negativos (BGN) apresentando o fenótipo de multirresistência ou extensivamente resistente são muito diversificados, e incluem: inativação/alteração da droga, alteração do sítio ativo, hiperexpressão de bombas de efluxo, sistema de modificação de lipopolissacarídeo (LPS) e perda de porina. Esses mecanismos estão associados com elementos genéticos móveis ou não, tais como integrons, transposons e plasmídeos, que podem facilitar a captura, acumulação e disseminação de genes de resistência. Assim, esses mecanismos exclusivos ou combinados, somados a escassez de antimicrobianos disponíveis no

momento, e a importância epidemiológica de algumas linhagens de BGN, tornaram esses microrganismos um grande desafio não apenas no ambiente hospitalar.

A presente tese de Doutorado foi realizada no Laboratório de Microbiologia Molecular do Instituto de Ciências Biomédicas da Universidade Federal de Uberlândia – UFU. Embora a maioria das metodologias utilizadas nesse estudo tenham sido empregadas nesse laboratório, a equipe contou com a colaboração fundamental de outros laboratórios, com destaque para o Laboratório de Microrganismos do Cerrado (LAMICE) (UFU), Laboratório de Genética (UFU) e Centro de Referência Nacional em Dermatologia Sanitária e Hanseníase (UFU). Adicionalmente, a Comissão de Controle de Infecção Hospitalar (CCIH) da UFU também teve importante papel no auxílio de dados demográficos e microbiológicos de pacientes no Hospital da Universidade referida.

Esse trabalho teve como objetivos principais: (i) avaliar a epidemiologia de infecções de corrente sanguíneas (ICS) associadas à assistência à saúde em Unidades de Terapia Intensiva (UTIs) causadas pelas principais três espécies de Bacilos Gram-negativos (*A. baumannii*, *K. pneumoniae* e *P. aeruginosa*) no hospital de clínicas da Universidade Federal de Uberlândia; (ii) avaliar os fatores de risco bem como como o impacto da terapia inapropriada nas infecções mencionadas acima; (iii) verificar a presença de genes de virulência e resistência de cepas de *K. pneumoniae* resistentes aos carbapenêmicos recuperadas de diferentes hospitais e regiões da cidade de Uberlândia no ano de 2020; (iv) avaliar a disseminação de clones de alto risco entre as linhagens de *K. pneumoniae* resistentes aos carbapenêmicos recuperadas de diferentes regiões da cidade de Uberlândia (norte, sul, centro, leste e oeste) no ano de 2020;

Esta tese foi subdividida em cinco capítulos, conforme apresentado a seguir:

Capítulo I – Revisão Bibliográfica - Apresenta uma revisão de literatura atualizada sobre ICS relacionadas à assistência à saúde (IRAS) causadas pelos principais BGN (*A. baumannii*, *K. pneumoniae, P. aeruginosa*) em especial cepas de *K. pneumoniae* resistentes aos carbapenêmicos.

Capítulo II - Bloodstream infections caused by multiresistant Gram negative bacilli in an intensive care unit: patient outcome and the impact of inappropriate empiric antibiotic therapy. Artigo submetido para publicação no

periódico São Paulo Medical Journal (Fator de Impacto 1.838, Qualis CAPES A4 - Ciências Biológicas III) em 2023, descrevendo a epidemiologia de ICS causadas por bacilos Gram-negativos na Unidade de Terapia Intensiva da Universidade Federal de Uberlândia, com altas frequências de ICS causadas por *A. baumannii*, *P. aeruginosa* e *K. pneumoniae* multirresistentes. Adicionalmente, pacientes com infecção causadas por estes microrganismos têm pior prognóstico principalmente quando a terapia antimicrobiana é considerada inadequada.

Capítulo III - A decade-long retrospective study of bloodstream infections caused by carbapenem-resistant Gram-negative bacilli in an Intensive Care Unit at a prominent referral hospital in Brazil. Artigo submetido para publicação no periódico Revista da Sociedade Brasileira de Medicina Tropical (Fator de Impacto 1.581, Qualis CAPES B1 - Ciências Biológicas III) em 2023, apresentando um estudo retrospectivo de 10 anos (2010-2019) de infecções causadas por *A. baumannii*, *P. aeruginosa* e *K. pneumoniae* resistentes aos carbapenêmicos, bem como a detecção de surtos no período estudado.

Capítulo IV - Exploring Virulence Genes, Carbapenemase-Encoding Genes, and Clonality in Carbapenem-Resistant *Klebsiella pneumoniae* Isolates: Insights from Public Hospitals in the Southeast Region of Brazil. Artigo será submetido para publicação no Journal of Medical Microbiology (Fator de Impacto 3.196, Qualis CAPES A4 - Ciências Biológicas III) em 2023, retratando um estudo prospectivo na cidade de Uberlândia, a respeito de infecções causadas por *K. pneumoniae* nos principais centros médicos públicos. No estudo foi pesquisado a presença dos principais genes codificadores de carbapenemases, bem como genes de virulência e adicionalmente foi realizada a análise de perfil clonal das cepas coletadas no período do estudo.

Capítulo V – Considerações finais. Principais conquistas obtidas com o estudo, contribuições e aplicabilidade dos resultados da pesquisa.

Capítulo I

Fundamentação teórica

1. Infecções relacionadas à assistência à saúde (IRAS)

As infecções relacionadas à assistência à saúde (IRAS) representam um sério risco para os pacientes, sendo o evento adverso mais comum nos cuidados de saúde (WHO, 2016). Estas infecções são especialmente graves sendo responsáveis por causarem índices elevados de morbidade, mortalidade e custos, tanto em países desenvolvidos quanto naqueles em desenvolvimento (IORDANO et al., 2020; RANGELOVA et al., 2020; SAX et al., 2020; SCAMARDO et al., 2020). Nas Unidades de Terapia Intensiva (UTIs), a literatura mostra que o problema pode ser mais grave ainda (VINCENT et al., 2009; SILVA et al., 2012; ILIYASU et al., 2016). No entanto, as IRAS ainda não receberem a atenção adequada, apesar do impacto negativo significativo na saúde coletiva (SCHERBAUM et al., 2014). Estima-se que em países desenvolvidos, a taxa de incidência de IRAS em pacientes na UTI é de 13,9 em 1000 pacientes/dia, enquanto em países subdesenvolvidos essa taxa sobre para 47,9 por 1000 pacientes/dia (ALLEGRANZI et al., 2011).

Em países de baixa e média renda como o Brasil, as taxas de IRAS principalmente aquelas causadas por microrganismos multirresistentes são significativamente mais altas que aquelas em países desenvolvidos (PADOVEZE; FORTALEZA, 2014). Essa disparidade é geralmente atribuída a escassez de recursos financeiros e humanos, infraestrutura inadequada nos serviços de saúde, incluindo falta de laboratórios de microbiologia habilitados, medidas insuficientes de prevenção e controle de IRAS, além do uso abusivo e indiscriminado de antimicrobianos (GIRIJALA; BUSH, 2017).

Quando se trata de IRAS, no estudo de Fortaleza e colaboradores (2017), realizado entre novembro de 2011 e abril de 2013, envolvendo instituições hospitalares de 10 estados do Brasil, a taxa de infecção de um modo geral foi de 10,8%, sendo a região Norte com a maior prevalência de IRAS (12,2%), seguida pelas regiões Centro-Oeste (11,3%), Sudeste (10,2%), Nordeste (9,3%) e Sul (8,3%). No mesmo estudo foi possível constatar que a pneumonia foi a infecção mais comum (3,6%), seguida por infecções de corrente sanguínea (2,9%), infecções do sítio cirúrgico (1,5%) e infecções do trato urinário (1,4%) (FORTALEZA et al., 2017).

No âmbito epidemiológico, as infecções causadas por bactérias multirresistentes (que são resistentes a um ou mais agentes antimicrobianos em três ou mais categorias) (MAGIORAKOS et al., 2012) estão relacionadas a vários fatores de risco. Esses fatores incluem o uso prévio de antimicrobianos, presença de comorbidades como Diabetes *mellitus*, uso de procedimentos invasivos como ventilação mecânica, cateter venoso central (CVC) e cateter urinário, tempo prolongado de hospitalização (igual ou superior a 45 dias) entre outros fatores intrínsecos e extrínsecos (ARAÚJO et al., 2016; FOUNOU et al., 2017; PATOLIA et al., 2018). No estudo realizado por Almeida Junior e colaboradores (2023), em 35 UTIs do estado de Minas Gerais no ano de 2016, foram identificados os seguintes fatores de risco: tempo de internação prolongado em UTI, uso de CVC por um período igual ou superior a 7 dias, uso de ventilação mecânica, uso de terapia antimicrobiana, uso de carbapenêmicos, cefalosporinas (terceira ou quarta geração) e polimixina.

O uso inadequado de antibióticos é uma prática amplamente difundida em todo o mundo, independentemente do nível de renda dos países (SLEATH et al., 2001; CURCIO et al., 2009; ALLEGRANZI et al., 2011). Ao longo das últimas oito décadas, o uso excessivo de antibióticos de forma inadequada, tem contribuído para o aumento da incidência e disseminação de bactérias resistentes, além de como mencionado anteriormente, uma maior morbidade, mortalidade e custos (CURCIO et al., 2009; ILIYASU et al., 2016; CHEREAU et al., 2017; BESHAH et al., 2022).

De acordo com o CDC (*Centers for Disease Control and Prevention*), nos Estados Unidos, a resistência aos antimicrobianos pode aumentar em cerca de US$ 1.400 o custo hospitalar para o tratamento de pacientes com infecções bacterianas (CDC, 2015). Em estudo conduzido por Osme e colaboradores (2020) em um hospital de ensino terciário de referência no Brasil, com o objetivo de analisar os custos de internação e os custos diretos relacionados às IRAS em pacientes na UTI, foi possível constatar que pacientes com IRAS apresentam um tempo de internação mais longo (15 dias) com custo médio por hospitalização de US$ 2.721, o que representou um aumento de 75% em comparação com pacientes sem IRAS, cujo custo foi de US$ 1.553. Especificamente na UTI, a ocorrência de IRAS está associada a um custo direto total oito vezes maior em comparação com pacientes sem infecções nessa unidade, sendo US$ 11.776 versus US$ 1.329, respectivamente (OSME et al, 2020).

Ainda no referido estudo citado acima, dentre as IRAS causadas pelos principais fenótipos de resistência, observou-se que as infecções provocadas por *P. aeruginosa* e *A. baumannii* resistentes ao carbapenêmicos apresentaram um aumento de US$ 5.460, enquanto no caso de infecções causadas por *K. pneumoniae* resistente aos carbapenêmicos, o aumento foi de US$ 5.033 (OSME et al, 2020).

2. Mecanismos de Resistência em Bacilos Gram-negativos

É consenso que a emergência e disseminação de bactérias multirresistentes tornou-se um problema de saúde pública globalizada, resultado direto do uso abusivo e irracional de antimicrobianos (GONTIJO-FILHO et al., 2016; RIBAS et al., 2021). A resistência aos antimicrobianos, especialmente em bacilos Gram-negativos (BGN), limita as opções terapêuticas eficazes disponíveis, consequência da habilidade desses microrganismos em desenvolver resistência a todos antimicrobianos disponíveis para tratamento (HENDRIKSEN et al., 2019).

Estudos recentes na região sudeste do Brasil revelaram um elevado uso de antimicrobianos nos hospitais, especialmente nas UTIs (MOREIRA et al., 2013; ROSSI et al., 2016; DANTAS et al., 2017; BRAGA, 2019). O uso empírico de antimicrobianos também foi frequente, com ênfase no uso de carbapenêmicos e antimicrobianos de amplo espectro, como cefalosporinas e mais preocupante ainda o uso de polimixinas, em hospitais universitários e não universitários (BRAGA, 2019).

Até o momento, já foram relatados mecanismos de resistência a todas as classes de antimicrobianos. Esse fato é extremamente importante, visto que é comum identificar em um mesmo microrganismo a presença de vários mecanismos que conferem resistência a diferentes classes de antimicrobianos, comprometendo drasticamente as opções terapêutica para o tratamento dessas infecções (BUSH, 2018). A Organização Mundial da Saúde (OMS) classifica espécies de BGN, como *P. aeruginosa*, *A. baumannii* e Enterobacteriaceae, que apresentam resistência a carbapenêmicos e β-lactamases de espectro estendido (ESBLs), como patógenos críticos e de alta prioridade (WHO, 2018).

A presença de ESBLs e carbapenemases são mecanismos de destaque que conferem resistência aos beta-lactâmicos, incluindo as famílias das cefalosporinas, penicilinas e carbapenêmicos (LINCOPAN et al., 2006; BUSH, JACOBY, 2009). As

ESBLs são comumente encontradas em diferentes membros da família Enterobacteriaceae, assim como em alguns bacilos Gram-negativos não-fermentadores. No entanto, *K. pneumoniae* e *Escherichia coli* são os principais microrganismos produtores de ESBL (PITOUT et al., 2005).

Na América do Sul, especialmente no Brasil, as ESBLs predominantes pertencem à família CTX-M, que podem ser agrupadas em cinco grupos com base em suas sequências de aminoácidos (ROCHA; PINTO; BARBOSA, 2016). Vários fatores contribuem para o sucesso dessas ESBLs, incluindo a capacidade de captura e disseminação do gene *bla*$_{\text{CTX-M}}$ por elementos genéticos móveis, a associação com clones bacterianos de alto risco e a pressão seletiva exercida pelo uso indiscriminado de cefalosporinas de amplo espectro e fluoroquinolonas em ambientes clínicos e veterinários (D'ANDREA et al., 2013).

Além das enzimas ESBLs da família CTX-M, outras beta lactamases como TEM e SHV, também estão associadas a surtos hospitalares significativos (CANTÓN et al., 2012). Por isso, os carbapenêmicos são frequentemente utilizados como opção terapêutica para tratar infecções graves causadas por microrganismos produtores de ESBLs (TAMMA; RODRIGUEZ BAÑO, 2017).

Já as carbapenemases são as β-lactamases mais potentes uma vez que essas enzimas têm a capacidade de hidrolisar imipenem, meropenem, outras penicilinas e cefalosporinas (BERTONCHELI; HÖRNER, 2008; PITOUT; NORDMANN; POIREL, 2015). As carbapenemases do tipo KPC (*K. pneumoniae* produtoras de carbapenemases do tipo KPC-2) são comumente encontradas em *K. pneumoniae*, mas também foram relatadas em outros microrganismos da família Enterobacteriaceae, bem como em bacilos Gram-negativos não fermentadores, como *P. aeruginosa* e *A. baumannii* (CHEN et al., 2014; PITOUT; NORDMANN; POIREL,2015; AL-RASHED et al., 2023; CAMARGO et al., 2023; KIFFER et al., 2023).

Além da KPC, outras carbapenemases têm assumido importância epidemiológica devido à sua associação com transposons, plasmídeos e integrons (PARTRIDGE et al., 2018). As metalo-β-lactamases (MBL) são destacadas nesse contexto, incluindo IMP (*Imipenemase*), VIM (*Verona Imipenemase*), SPM-1 (*São Paulo MBL*), GIM (*German Imipenemase*), SIM-1 (*Seul Imipenemase*), AIM-1

(*Australian Imipenemase*), KHM (*Kyorin University Hospital*), NDM-1 (*New Delhi MBL*) e DIM-1 (*Dutch imipenemase*) (MENDES et al., 2006; YONG et al., 2007; SEKIGUCHI et al., 2008; YONG et al., 2009; POIREL et al., 2010). Interessantemente, além da SPM-1 a enzima BKC-1 (*Brazilian Klebsiella carbapenemase-1*) é a segunda carbapenemase originalmente descrita no Brasil, inicialmente descrita em isolados clínicos de *K. pneumoniae* do estado de São Paulo (NICOLETTI et al., 2015).

Além das carbapenemases mencionadas, as enzimas do tipo oxacilinases (OXA) também desempenham um papel importante nesses microrganismos. Embora tenham menor capacidade hidrolítica, essas enzimas não devem ser menosprezadas em termos de importância (PEREIRA et al., 2015). A disseminação ampla e rápida das carbapenemases tem impactado negativamente o tratamento das infecções causadas por BGNs aumentando significantemente as taxas de mortalidade (POIREL; JAYOL; NORDMANN, 2017).

A dificuldade nesse tratamento de infecções graves causadas por esses microrganismos multirresistentes levou à reintrodução das polimixinas (polimixina B e colistina) como última opção terapêutica em todo o mundo (POIREL; JAYOL; NORDMANN, 2017). No entanto, o aumento do uso desses antimicrobianos tem levado ao surgimento de resistência às polimixinas em bactérias que normalmente são suscetíveis a essas drogas (OLIVEIRA et al., 2018). Isso representa uma grande ameaça, uma vez que estamos diante de infecções intratáveis, onde o aspecto mais preocupante é a capacidade desses microrganismos de transferir genes de resistência para outras espécies bacterianas, o que favorece ainda mais esse cenário (WANG et al., 2018).

Entre as Enterobacteriaceae resistentes aos carbapenêmicos e as cefalosporinas de amplo espectro, priorizadas pela OMS para pesquisa e desenvolvimento de antimicrobianos novos, a *K. pneumoniae* é um microrganismo altamente versátil, reconhecido por sua capacidade de causar infecções hospitalares e infecções adquiridas na comunidade em todo o mundo, apresentando alta taxa de mortalidade em pacientes gravemente doentes, possivelmente relacionada à administração inadequada de tratamento antimicrobiano (CHEN et al., 2013; MARTIN; BACHMAN, 2018; KO et al., 2002; BENGOECHEA; PESSOA, 2018; WHO, 2018)

A *K. pneumoniae* emergiu como um microrganismo multirresistente de destaque, responsável por IRAS, devido à gravidade das infecções e à escassez de tratamentos eficazes disponíveis, e apesar dos avanços na compreensão dos aspectos microbiológicos, clínicos e epidemiológicos, ainda há lacunas no conhecimento dos reservatórios ambientais dos genes de resistência (CARATTOLI, 2017; MARTIN; BACHMAN, 2018; LARSSON; FLACH, 2022; GAJIC et al., 2023).

No ambiente hospitalar, a presença desse microrganismo tem sido amplamente documentada. O estudo do Programa de Vigilância Antimicrobiana SENTRY (SENTRY), realizado na América Latina de 2008 a 2010, revelou que a *K. pneumoniae* é uma das principais causadoras de infecções sanguíneas, representando 12,3% dos casos, ficando logo atrás da *E. coli* (19%). Em um estudo desenvolvido em São Paulo que avaliou surtos de IRAS em um período de três anos, constatou-se que as infecções causadas por *K. pneumoniae* foram as mais prevalentes (DE JESUS MARÇAL et al., 2020). O mesmo resultado também foi obtido em estudo desenvolvido em dois hospitais Pelotas (Rio Grande do Sul) em um período de quatro meses que avaliou infecções de pacientes internados e atendidos no pronto socorro (JARA et al., 2021) e também em Salvador (Bahia), que avaliou pacientes com ICS em um período de 1 ano (LEAL et al., 2019), evidenciando a presença de *K. pneumoniae* como um principal agente causador de infecções no ambiente hospitalar.

Estima-se que, do ponto de vista epidemiológico, cerca de 50% das cepas de *K. pneumoniae* sejam produtoras de ESBL no Brasil (VILLEGAS et al., 2011). Essa alta prevalência traz consigo um aumento no risco de falhas terapêuticas e de mortalidade, impactando significativamente a saúde pública global. Adicionalmente, o uso frequente de carbapenêmicos tem levado à seleção de cepas resistentes, incluindo as produtoras de carbapenemases, como a KPC e as MBL (VAN BOXTEL et al., 2016).

No Brasil, cepas de *K. pneumoniae* produtoras de KPC foram relatadas pela primeira vez em 2009, em Recife, mas estudos posteriores mostraram que essas cepas já existiam desde 2005 em São Paulo (MONTEIRO et al., 2009; PAVEZ et al., 2009). Desde então, a disseminação endêmica dessas cepas tem sido documentada em todo o país, associada a surtos de infecções hospitalares (GALES et al., 2012; PEREIRA et al., 2013).

Quando se trata de patógenos como *A. baumannii*, *E. coli*, *K. pneumoniae* e *P. aeruginosa*, existe um interesse amplo em estudar como ocorre a disseminação de tantos genes de resistência bem como o estabelecimento de clones altamente adaptáveis no ambiente. A literatura mostra a participação importante e decisiva dos plasmídeos, transposons e integrons nesse processo, particularmente associados a presença de genes que codificam β-lactamases de espectro estendido e carbapenemase como CTX-M e KPC, respectivamente (ORLEK et al., 2017; PASKOVA et al., 2018; RUI et al., 2018; CERDEIRA et al., 2019; REYES et al., 2023).

K. pneumoniae possui uma ampla variedade de mecanismos de resistência, incluindo inativação e alteração do fármaco, alteração do sítio ativo, hiperexpressão de bombas de efluxo, modificação do lipopolissacarídeo (LPS) e perda de porina, sendo esses mecanismos frequentemente associados a elementos genéticos móveis, como integrons, transposons e plasmídeos, que facilitam a aquisição, acumulação e disseminação de genes de resistência (NAVON-VENEZIA; KONDRATYEVA; CARATTOLI, 2017; Partridge et al., 2018).

Como apresentado até agora, mundialmente, as infecções causadas por fenótipos multirresistentes principalmente por bactérias Gram-negativas ainda são responsáveis por causarem inúmeros prejuízos a saúde, sendo a resistência um dos pontos chaves desse problema (VILLEGAS et al., 2011; HORMOZI et al., 2018). Entretanto, associado a isso, diversos fatores de virulência também contribuem para os processos de adaptação dos microrganismos, favorecendo aspectos relacionados com o alto grau de patogenia dos microrganismos (RUSSO et al., 2010)

Assim, o estudo e a identificação de fatores de virulência e o conhecimento de como esses fatores se manifestam e/ou interagem podem elucidar o entendimento de como esses fatores contribuem para a evolução do processo infeccioso nos pacientes. Em microrganismos Gram-negativos, a literatura tem mostrado grande quantidade e variedade de fatores de virulência, dentre esses fatores podemos destacar a importância das pílis, cápsulas, sideróforos, adesinas, formação de biofimes e entre outros (SHON et al., 2013; LI et al., 2019).

Nesse trabalho, foram buscados genes que codificam fatores de virulência como mecanismos de sistemas de aquisição de ferro, como enterobactina (*entb*), que medeiam

a absorção de ferro férrico (*kfu*) e o gene *allS* associado ao metabolismo da alantoína, sendo a presença destes mais prevalentes em cepas hipervirulentas (MA et al., 2005; BACHMAN et al., 2011; BIALEK-DAVENET et al., 2014).

A investigação da epidemiologia das IRAS causadas por bactérias multirresistentes tem passado por avanços significativos na área da tipagem molecular, o que permite comparar bactérias em nível global e identificar a ocorrência de clones emergentes (PÉREZ-LOSADA et al., 2013). Esses avanços têm ampliado nossa compreensão sobre a mobilização de genes e a ameaça crescente da resistência aos antimicrobianos (MATHERS; PEIRANO; PITOUT, 2015).

Clones multirresistentes de alto risco apresentam características distintas que os qualificam, incluindo: (i) distribuição global, (ii) associação com múltiplos determinantes de resistência antimicrobiana, (iii) habilidade de colonizar e persistir em hospedeiros por longos períodos de tempo, (iv) eficiente capacidade de transmissão entre hospedeiros, (v) maior patogenicidade e adaptabilidade, e (vi) potencial para causar infecções graves e/ou recorrentes (MATHERS; PEIRANO; PITOUT, 2015).

K. pneumoniae produtora da enzima KPC-2, pertencente ao complexo clonal 258 (CC258), tem se tornado globalmente predominante devido à sua alta capacidade de transmissão (CHEN et al., 2014; PITOUT; NORDMANN; POIREL, 2015). Esse complexo clonal é composto principalmente pelo sequence type (ST) 258, encontrado com frequência na América Latina, América do Norte e Europa, enquanto o ST11 é mais prevalente na América Latina e Ásia (CHEN et al., 2014). Há indícios que o CC258 se espalhou rapidamente pelo mundo de maneira semelhante ao CC17 de *Enterococcus faecium* resistente à vancomicina (VRE) e CC22 de *A. baumannii* resistente a carbapenem (CRAB) (WILLEMS et al., 2005; FU et al., 2010). No Brasil, os ST11, ST340 e ST437 são considerados endêmicos (PEREIRA et al., 2013; SHEN et al., 2016).

Considerando os aspectos apresentados até agora, fica evidente que o esforço para compreender a epidemiologia das infecções causadas por BGN multirresistentes. Além disso a aplicação de técnicas moleculares permite aumentar nossa compreensão sobre a emergência, reemergência e disseminação desses fenótipos, colaborando de forma ímpar no estabelecimento de estratégias de prevenção e controle dessas infeções.

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Capítulo II

Bloodstream infections caused by multiresistant Gram negative bacilli in an Intensive Care Unit: patient outcomes and the impact of inappropriate empiric antibiotic therapy

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ORIGINAL ARTICLE

Bloodstream infections caused by multiresistant Gram negative bacilli in an intensive care unit: patient outcomes and the impact of inappropriate empiric antibiotic therapy.

Vinícius Lopes Dias[a], Sebastiana Sabino Silva[a], Melina Lorraine Ferreira[a], Jane Eire Uzredo Cunha[a], Iara Rossi Gonçalves[a], Bruna Fuga Araújo[b], Luiz Gustavo Machado[a], Paulo Pinto Gontijo Filho[a], Rosineide Marques Ribas[a]*.

[a]Institute of Biomedical Sciences, Laboratory of Molecular Microbiology, Federal University of Uberlândia (UFU), Uberlândia (MG), Brazil.
[b]School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

*Corresponding author:
E-mail: rmribas@ufu.br (Ribas, RM)
Tel.: +55 34 32258574
Present Address: Laboratório de Microbiologia - Instituto de Ciências Biomédicas, Av. Amazonas, S/N, Bloco 2B, Sala 214 (Piso Superior), Campus Umuarama, CEP/Zip Code: 38400-902. Uberlândia – MG, Brazil.

Abstract

Background: Bloodstream infections (BSI) caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Acinetobacter baumannii* are challenging. **Objective:** This study was conducted to elucidate the risk factors associated with the mortality of patients with BSI caused by these three GNB species, and to assess the potential impact of inappropriate empirical antibiotic therapy on patient mortality. **Design and setting:** We carried out a retrospective analysis involving a cohort of 381 patients who experienced their initial episode of BSI caused by *P. aeruginosa, K. pneumoniae*, and *A. baumannii* bacteremia in an adult Intensive Care Unit (ICU) between January 2011 and December 2019. **Methods:** The investigation was conducted utilizing an anonymous questionnaire, and standard information was gathered from the medical records of the patients, focusing on their underlying health conditions. Additionally, microbiological data related to these infections were also evaluated. **Results:** A total of 257 patients (67.4%) with

bacteremia attributed to carbapenem-resistant microorganisms were identified, with *A. baumannii* being the most prevalent pathogen (51.8%). Overall, 50.1% of the patients exhibited infections caused by multiresistant bacteria. The mortality rates for BSI attributed *to A. baumannii, K. pneumoniae*, and *P. aeruginosa* were 53.2%, 42.2%, and 63.2%, respectively. Notably, patients with BSI caused by MDR microorganisms exhibited a more unfavorable prognosis when compared to those with infections caused by non-multidrug resistant microorganisms. **Conclusions:** Our data underscores a concerning prevalence of carbapenem-resistant microorganisms and MDR isolates in BSI caused by *A. baumannii, P. aeruginosa*, and *K. pneumoniae*. These occurrences are frequently associated with unfavorable patient outcomes and instances of inappropriate therapeutic approaches.

Keywords: Intensive care units; Bloodstream Infections; Gram-negative bacterial infections, Multiple Drug Resistance.

Introduction

Bloodstream infections (BSI) caused by Gram negative bacilli (GNB) have become as escalating challenge globally, particularly within Intensive Care Units (ICU)[1]. According to the World Health Organization (WHO), GNB as Carbapenem-resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were recognized as critical-priority, mainly because these are associated to a high mortality and high costs[2].

It is estimated that in low and middle-income countries, such as Brazil, 51.6% of patients admitted to ICU acquire at least one healthcare-associated infection (HAI), with around 56.1% of these infections being BSI caused by multidrug-resistant (MDR) GNB. These infections are progressively emerging as more prevalent and consequential within healthcare settings, particularly among critically ill patients in ICUs[3,4]. *A. baumannii, K. pneumoniae* and *P. aeruginosa* are the primary perpetrators of these infections, and they often exhibit resistance to multiple antibiotics, presenting a formidable challenge for effective treatment[5-9].

Objective

The current study was conducted to identify risk factors associated with the mortality of patients in the ICU who have BSI caused by *K. pneumoniae, P. aeruginosa*, and *A. baumannii.* Additionally, the study aims to examine how inappropriate empirical antibiotic therapy contributes to these deaths.

Methods

Settings, patient cohort, study design and data collection

This was a retrospective cohort study of BSI caused by *P. aeruginosa, A. baumannii*, and *K. pneumoniae* carbapenem resistant microorganisms in adult patients admitted to the ICU of a teaching hospital between 2011 and 2019. This study was conducted at Uberlândia University Hospital, a 530-bed teaching and referral hospital situated in Brazil. Data collection for this study utilized the bacteremia records from the hospital's Microbiology Laboratory. The study specifically concentrated on cases of nosocomial bacteremia that were acquired within the ICU. Only patients who had been hospitalized in the ICU for at least 48 hours prior to the diagnosis of bacteremia were deemed eligible for inclusion in this study.

A retrospective study design was utilized to identify predictors of mortality and assess the clinical implications of resistance, along with the consequences of inappropriate therapy. The primary outcome measure examined in this study was in-hospital mortality.

The study utilized an anonymous questionnaire and standard information was collected from medical records. This included demographics, clinical characteristics (such a illness severity, comorbidities), presence of invasive devices, length of total hospital stay, length of ICU stay, and invasive procedures. The medical history of the patient’s underlying conditions, such as diabetes mellitus, chronic renal failure, heart failure, Acquired Immuno-Deficiency Syndrome (AIDS), and cancer, were determined. We also assessed the sources of bacteremia, previous antibiotic use during current hospitalization, and cases of inadequate antimicrobial treatment.

Microbiology investigations

Microbial identification and antimicrobial susceptibility testing were performed on the

Vitek-2 system (bioMérieux, Marcy l'Etoile, France). Antibiotics tested included aminoglycosides (gentamicin, amikacin), carbapenems (imipenem, meropenem), cephalosporins (cefepime), fluoroquinolones (ciprofloxacin) and b-lactamase inhibitors (piperacillin-tazobactam). Susceptibilities were determined according to the standards of the Clinical and Laboratory Standard Institute (CLSI). Isolates with intermediate susceptibility were considered as resistant.

Definitions

According to the Centers for Disease Control (CDC), bacteremia is defined as the presence of viable micro- organisms in the bloodstream documented by a positive blood culture result. Bacteremia was considered to be nosocomial if the infection occurred >48 h after admission and no clinical evidence of infection on admission existed[6]. Bacteremia was classified as primary when the patient had a recognized pathogen cultured from one or more blood cultures and the organism cultured from blood was not related to an infection at another site. Catheter-related bacteremia was defined as when the patient had an intravascular device and one or more positive blood culture results were obtained from the peripheral vein, and there was no apparent source for infection except the catheter. Bacteremia was considered secondary when the patient had a recognized pathogen cultured from one or more blood cultures and the organism cultured from blood was related to an infection at another site[8].

Multidrug-resistant Gram-negative bacteria (MDRGN) was defined as Enterobacteriaceae, *A. baumannii*, or *P. aeruginosa* with resistance against at least three out of four antibiotic classes[9].

Antimicrobials were considered empirical if they were administered before susceptibility test results were available (typically, 48 to 72 h after blood cultures were performed) and were considered definitive thereafter. Empirical therapy was considered adequate when all the following criteria were fulfilled: at least one antimicrobial was administered as recommended, the organism isolated from blood were susceptible *in vitro*; and the first dose was administered within the first 24 h after the blood culture had been drawn. Otherwise, empirical therapy was considered inadequate.

Statistical analysis

Student's *t*-test was used to compare continuous variables and χ^2 or Fisher's exact test was used to compare categorical variables. Variables with $P \leq 0.05$ in the univariate analysis were candidates for multivariate analysis. A survival curve was constructed using the Kaplan–Meier method, and the log-rank test was used for comparisons between patients with inappropriate and adequate therapy. All *P* values were two-tailed and $P \leq 0.05$ was considered to be statistically significant. The analyses were carried out using the GraphPad Prism software package (La Jolla, CA, EUA), and BioEstat 5.0 (Tefé, AM, Brasil).

Ethical approval

The study was approved by the Committee of the Federal University of Uberlandia (228/11). Since this study did not involve experimentation on human subjects, written informed consent was waived by our institutional review board. The patient information used in this study was anonymized before its use.

Results

This study included a total of 381 non-repeat bacteremia events resulting from *P. aeruginosa, A. baumannii*, or *K. pneumoniae* during study period. Of these bacteremia cases, *K. pneumoniae* accounted for 109 (28.6%), *P. aeruginosa* for 114 (29.9%), and *A. baumannii* for 158 (41.5%).

Data on carbapenem susceptibility were collected and presented in Tables 1, 2 and 3. Among the bacteremia cases, 257 were caused by a carbapenem-resistant isolate, with an overall prevalence of carbapenem resistance of 67.4%. *A. baumannii* had the highest carbapenem resistance rate (84.2%) and caused 51.8% of all carbapenem-resistant bacteremia. Multirresistance were observed in 50.1 % of the total microorganisms and the most prevalent MDR microorganism was *A. baumannii* (80.6%) with *K. pneumoniae* and *P. aeruginosa* showing high resistance rates.

Of the 381 ICU patients selected for the study, 158 (41.5%) developed BSI caused by *A. baumannii*, where 74 (46.8%) within this group died. Most of these patients were male (69.6%) with a mean age of 54.0 years old. Among the comorbidities, the most frequent was the presence of heart failure (34.8%), with 24.0% of these patients having

a Charlson index ≥3. As for invasive procedures, there was a high prevalence of central venous catheter (98.7%), vesical probe (98.1%) and tracheostomy (58.9%). The main source of these bacteremias was the respiratory tract (31.6%), and it was not possible to discover the source for 60.1% of these. In 87.3% of the patients, antimicrobials were administered empirically, and in 70.9% it was classified as inappropriate and/or inadequate, with the prevalent use of carbapenems and third and fourth generation cephalosporins. *A. baumannii* MDR strains were detected in 80.6% of infections (Table 1).

The key characteristics of the survivor and non-survivor subgroups are outlined in Table 1. According to the findings, the use of a tracheostomy (p=0.02) and hemodialysis catheter (p=0.02) showed statistically significant results in both multivariate and univariate analyses. Additionally, the presence of heart failure (p=0.003), malignancy (p=0.02), and diabetes mellitus (p=0.001) demonstrated statistically significant results in the univariate analysis.

From the total of 381 patients with BSI, 109 (28.6%) developed BSI caused by *K. pneumoniae.* Most of these patients were female with a mean age of 53.0 years old and among of the comorbidities, the most frequent was the presence of heart failure (41.3%). As for invasive procedures, there was a high prevalence of central venous catheter (88.1%) and tracheostomy (64.2%). The main source of these bacteremias was the respiratory tract (29.4%) with a notable prevalence of infections originating in the urinary tract (16.5%). In 72.2% of the patients, antimicrobials were administered empirically, and in 62.4% the antimicrobial therapy was deemed inappropriate and/or inadequate. *K. pneumoniae* MDR strains were detected in 25.7% of infections. Thirty days after BSI onset, 46 of the 109 patients (42.2%) had died (Table 2).

The main characteristics of the survivor and non-survivor subgroups are presented in Table 2, where according of the univariate analysis, the main risk factors were: patients coming from the medical clinic (p=0.048), using a vesical probe (p=0.009) and hemodialysis catheter (p=0.001). Regarding the use of antimicrobials, antimicrobial therapy was considered inappropriate (p=0.011). After selecting the statistically significant risk factors ($p\leq0.05$) and taken to multivariate analysis, only the presence of a vesical probe (p=0.003) and hemodialysis catheter (p=0.01) were shown to be statistically significant (Table 2).

Among the 381 patients with BSI, 114 (29.9%) developed BSI caused by *P. aeruginosa*. According to Table 3, the median age of the population was 54.0 years, and 86 (75.4%) patients were male. Regarding the underlying conditions, most presented at least one of these such: heart failure (32.5%), malignancy (21.0%) and diabetes mellitus (21.9%). Primary BSI and Respiratory tract infection were the most frequent sources of infection (44.7% and 43.9% respectively) (Table 3). As for invasive procedures, there was a high prevalence of central venous catheter (94.7%), vesical probe (88.6%) and tracheostomy (51.7%).

Most of the patients with BSI caused by *P. aeruginosa* were treated empirically and often in the wrong way and 33.3% of *P. aeruginosa* strains were classified as MDR. Thirty days after BSI onset, 42 patients (36.8%) had died. Other characteristics of the survivor and non-survivor subgroups are presented in Table 3.

The univariate analysis showed that several common risk factors in the literature were significant for each of the groups evaluated, however, the multivariate analysis showed that use of tracheostomy (p=0.02), use of hemodialysis catheter (p=0.02) and the empirical use of carbapenems (p=0.04) (for *A. baumannii*), use of vesical probe (p=0.003), use of hemodialysis catheter (p=0.01) (for *K. pneumoniae*) and use of hemodialysis catheter (p=0.01), inappropriate use of empirical therapy (p=0.02) and empirical use of carbapenems (p=0.05) (for *P. aeruginosa*) were independently associated with the worst outcome of these patients.

The survival distributions were significantly different (p=0.0463) in patients with BSI caused by these microorganisms MDR vs NMDR (Figure 1). Regarding the inappropriate and appropriate therapies in patients with BSI caused by MDR microorganisms, the 30-day survival distributions were significantly different in patients in BSI caused by *K. pneumoniae* and *P. aeruginosa* (Figure 2).

Discussion

Unfortunately, recent years have been reporting a significant worldwide increase in the frequency of healthcare associated infections caused by Carbapenem-resistant *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*, especially in ICUs.

In fact, studies conducted in low- and middle-income countries, such as Brazil, have

more frequently encountered Gram-negative isolates in blood cultures than Gram-positive isolates[10,11]. Heavy antibiotic use and inappropriate empirical therapy collaborated significantly so that the incidence of these pathogens have gradually increased in severe infections.

It is noteworthy that this study found an alarmingly high rate of carbapenem resistance among these three microorganisms in blood, with an overall prevalence of 67.4%, mainly in *A. baumannii* isolates (84.2%). Unfortunately, this finding represents calamity when it comes to the choice of the treatment, representing one of the biggest challenges now. Our data directly reflect what previous literature has also shown, such as the study of Agarwal *et al.*[12], that 90% of the *A. baumannii* isolates were carbapenem resistant.

In Brazil, because of the worryingly overuse and misuse of antimicrobials in the hospital setting, the emergence of multidrug-resistant isolates has continually increased over time, where the biggest consequence is that common infections cannot be treated[13]. Another fact that must be considered here is that the prevalence of dissemination of multidrug-resistant GNB has been critical, representing a considerable threat to public health. Corroborating with the study by Moges *et al.*[14], our data show extremely high rates of multidrug resistance, 50.1%.

In this scenario, studies establish a significant relationship between multiresistant strains and inappropriate antimicrobial therapy, which in the study conducted by Quillici *et al.*[15], 81.6% of the inappropriate empirical antibiotic therapy cases occurred with patients infected with antibiotic-resistant micro-organisms. In our study this rate was 58,8%. In short, our data reinforces the high frequency of Gram-negative bacteria in BSI, and that antimicrobial resistance is severe and worrisome in this hospital, being associated with worse prognosis.

Recently, several studies have shown that the inappropriate use of these antimicrobials is related to a worse prognosis for these patients. It was possible to examine the trend in mortality rate of patients for these three selected blood isolates, demonstrating an alarming increasing incidence over the study in this institution. These observed trends are alarming, considering that here, in Brazil, there is the overuse and inappropriate use of antimicrobials in the hospital setting[4,15].

Conclusion

In conclusion, the study data highlights an alarming frequency of carbapenem resistant microorganisms and MDR isolates in BSI by *A. baumannii*, *P. aeruginosa* and *K. pneumoniae*, which are often related to a bad patient prognosis. It is believed that the findings of this study will stimulate the use of efficient infection prevention and control measures, as well as antibiotic consumption by proper prescription.

Conflict of interest statement:

The authors have declared no conflict of interest.

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Legends of figures:

Figure 1: Kaplan-Meier curve for survival of BSI caused by MDR and NMDR *A. baumannii, K. pneumoniae and P. aeruginosa* in an ICU at the Hospital of the Federal University of Uberlândia (p=0.0463).

Figure 2. Kaplan-Meier curves for survival of inappropriate and appropriate therapies for BSI caused by GNB-MDR in patients in an ICU at the Hospital of the Federal University of Uberlândia. **a.** Curve for survival for survival of inappropriate and appropriate therapies for BSI caused by MDR *A. baumannii* (p=0.8789). **b.** Curve for survival of inappropriate and appropriate therapies for BSI caused by MDR *K. pneumoniae* (p=0.0471). **c.** Curve for survival of inappropriate and appropriate therapies for BSI caused by MDR *P. aeruginosa* (p=0.033).

Non-standard Abbreviations

AIDS: Acquired Immuno-Deficiency Syndrome

BSI: Bloodstream infections

CDC: Centers for Disease Control

CLSI: Clinical and Laboratory Standard Institute

GNB: Gram-negative-bacilli

HAI: Healthcare Associated Infection

ICU: Intensive Care Unit

MDR: Multidrug Resistant

MDRGN: Multidrug-resistant Gram-negative bacteria

NMDR: non - Multidrug Resistant

WHO: World Health Organization

Figure 1:

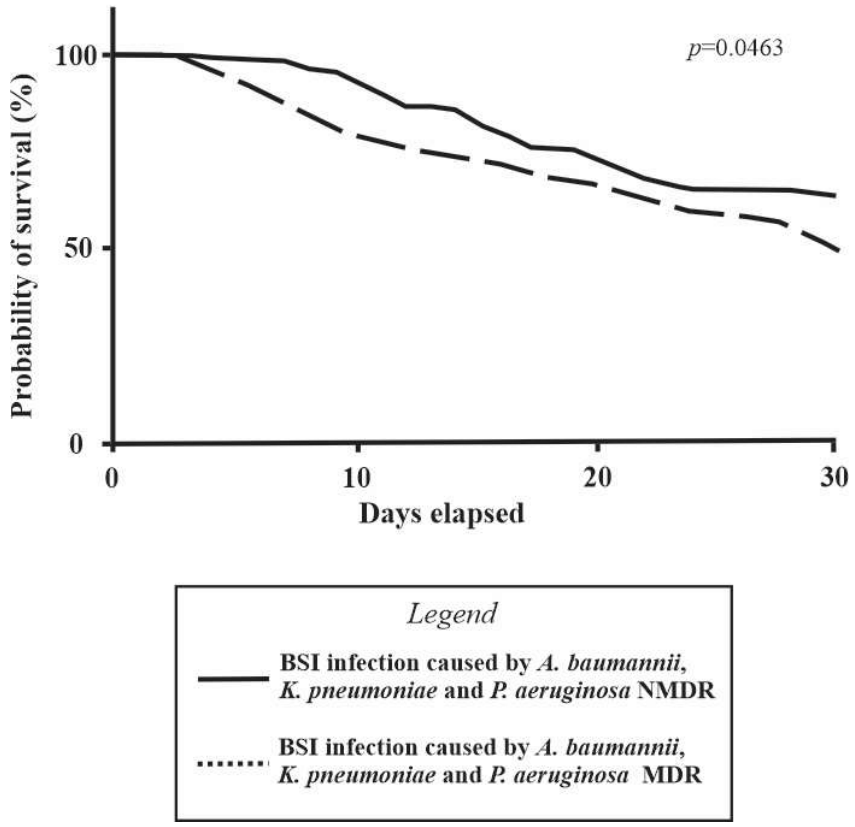

Figure 2:

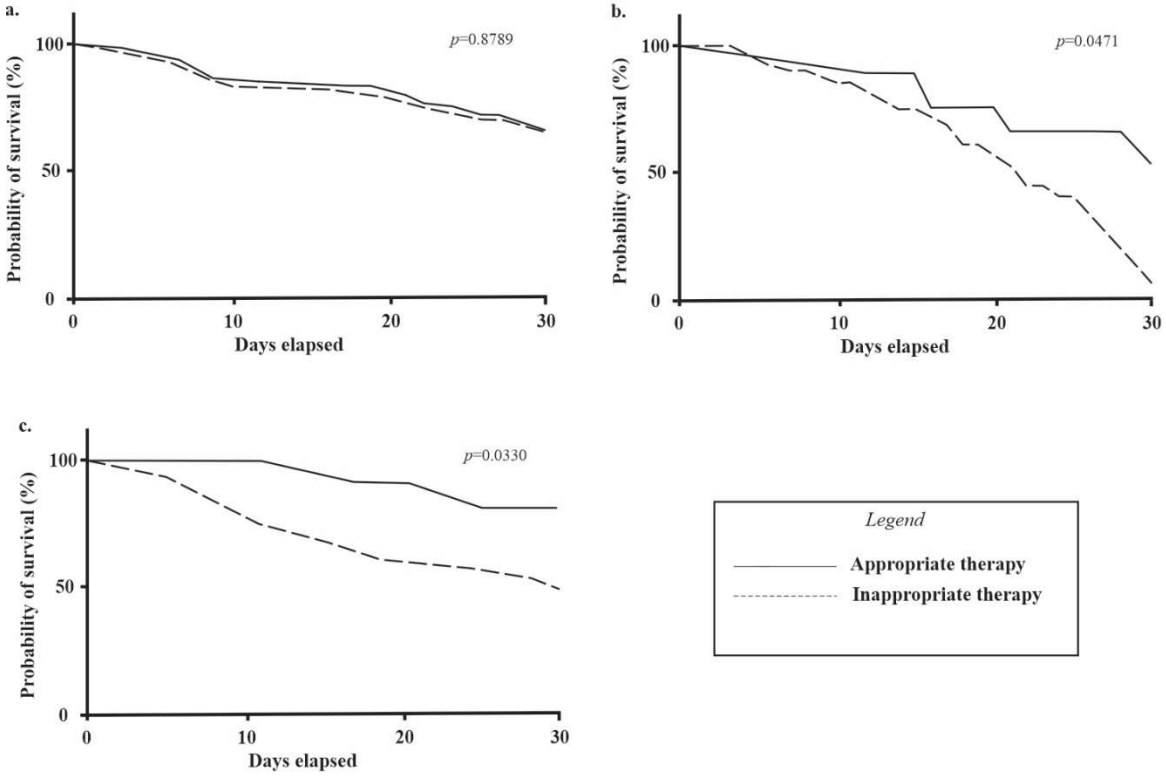

Table 1: Demographic, clinical and microbiological characteristics of patients with BSI caused by *Acinetobacter baumannii* in an ICU at the Hospital of the Federal University of Uberlândia (n = 158)

Characteristics	Total *n*[1] (%) *n* = 158 (%)	Survivors *n* (%) *n* = 84 (%)	Non-survivors *n* (%) *n* = 74 (%)	*Univariate*		*Multivariate*	
				p[2]	OR[3] (95% CI[4])	*p*	OR (95% CI)
Male	110 (69.6)	56 (66.7)	54 (73.0)	0.4	0.7 (0.4-1.5)	-	-
Hospital stay, days – mean	47.6	47.8	49.6	**0.025**	-	-	-
ICU[5] stay, days – mean	37.2	35	39.8	**0.006**	-	-	-
Age, years – mean	54	60.3	46.9	-	-	-	-
Patient origin							
Clinical	64 (40.5)	47 (55.9)	17 (23.0)	**<0.001**	**4.3 (2.1-8.5)**	**0.04**	**3.3 (1.0-10.0)**
Surgery	32 (20.2)	16 (19.0)	16 (21.6)	0.7	0.8 (0.4-1.8)	-	-
Traumatology		21 (25.0)	41 (55.4)	**<0.001**	**0.3 (1.3-0.5)**	0.6	1.4 (0.4 -4.7)
Comorbidity							
Heart failure	55 (34.8)	40 (47.6)	15 (20.3)	**0.003**	**3.6 (1.7-7.2)**	0.7	1.2 (0.4-3.4)
Nephropathy	21 (13.5)	14 (16.7)	7 (9.4)	0.2	1.9 (0.7-5.0)	-	-
Malignancy	24 (15.2)	18 (21.4)	6 (8.1)	**0.02**	**3.1 (1.1-8.3)**	0.3	1.9 (0.5-6.9)
Diabetes mellitus	18 (11.4)	16 (19.0)	2 (2.7)	**0.001**	**8.5 (1.9-38.2)**	0.08	4.5 (0.8-25.9)
Comorbidity index							
CHARLSON ≥3	38 (24.0)	29 (34.5)	9 (12.2)	**0.01**	**3.8 (1.7-8.7)**	0.6	1.3 (0.4-3.7)
Invasive procedures							
Central venous catheter	156 (98.7)	84 (100.0)	72 (97.3)	0.2	5.9 (0.3-123.5)	-	-
Mechanical ventilation	63 (39.9)	35 (41.7)	28 (37.8)	0.6	1.2 (0.6-2.2)	-	-
Vesical probe	152 (98.1)	80 (95.2)	72 (97.3)	0.7	0.5 (0.1-3.1)	-	-
Tracheostomy	93 (58.9)	42 (50.0)	51 (68.9)	**0.02**	**0.4 (0.2-0.9)**	**0.02**	**0.4 (0.1-0.5)**
Hemodialysis catheter	57 (36.1)	44 (52.4)	13 (17.6)	**<0.0001**	**5.2 (2.4-10.8)**	**0.02**	**2.9 (1.1-6.9)**

Table 1: Demographic, clinical and microbiological characteristics of patients with BSI caused by *Acinetobacter baumannii* in an ICU at the Hospital of the Federal University of Uberlândia (*continued*)

Characteristics	**Total** ***n*** **(%)** ***n*** **= 158 (%)**	**Survivors** ***n*** **(%)** ***n*** **= 84 (%)**	**Non-survivors** ***n*** **(%)** ***n*** **= 74 (%)**	*Univariate* ***p***	**OR (95% CI)**	*Multivariate* ***p***	**OR (95% CI)**
Source							
Primary	95 (60.1)	51 (60.7)	44 (59.5)	0.8723	1.054 (0.6-2.0)	-	-
Secondary	63 (39.9)	33 (39.3)	30 (40.5)	0.8723	0.9490 (0.5-1.8)	-	-
Urinary tract	6 (3.8)	3 (3.6)	3 (4.0)	0.1	0.9 (0.2-4.5)	-	-
Respiratory tract	50 (31.6)	25 (29.8)	25 (33.8)	0.6	0.8 (0.4-1.6)	-	-
Others	7 (4.4)	5 (5.9)	2 (2.7)	0.4	2.3 (0.4-12.1)	-	-
Prior use of antibiotics	138 (87.3)	73 (86.9)	65 (87.8)	0.9	0.9 (0.4-2.4)		
Inappropriate empirical therapy	112 (70.9)	65 (77.4)	47 (63.5)	**0.05**	**1.9 (0.9-3.9)**	0.8	1.1 (0.4-2.8)
Empirical use of cephalosporins	81 (51.3)	35 (41.7)	46 (62.2)	**0.01**	**0.4 (0.2-0.8)**	0.2	0.6 (0.2-1.4)
Empirical use of carbapenems	71 (44.9)	45 (53.6)	26 (35.1)	**0.02**	**2.1 (1.1-4.0)**	**0.04**	**2.3 (1.0-5.2)**
Empirical use of beta lactams	42 (26.6)	22 (26.2)	20 (27.0)	0.1	0.9 (0.5-1.9)	-	-
Empirical use of polimixins	4 (2.5)	3 (3.6)	1 (1.3)	0.6	2.7 (0.3-26.6)	-	-
A. baumannii MDR[6]	125 (80.6)	66 (78.6)	59 (79.7)	0.8	0.9 (0.4-2.0)	-	-
A. baumannii carbapenem resistant	133 (84.2)	73 (86.9)	60 (81.1)	0.3	1.6 (0.6-3.7)	-	-
Risk time (days-mean)	20	23.6	16	0.12	-	-	-
Risk time ICU (days-mean)	9.7	12.7	6.1	**0.029**	-	-	-

[1]*n*: number; [2]*p*: *P* value; [3]OR: Odds ratio; [4]CI: confidence interval; [5]ICU: Intense Care Unit; [6]MDR: multidrug resistant.

Table 2: Demographic, clinical and microbiological characteristics of patients with BSI caused by *Klebsiella pneumoniae* in an ICU at the Hospital of the Federal University of Uberlândia (n = 109)

Characteristics	Total *n*[1] = (%) *n* = 109 (%)	Survivors *n* (%) *n* = 63 (%)	Non-survivors *n* (%) *n* = 46 (%)	*Univariate*		*Multivariate*	
				p[2]	OR[3] (95% CI[4])	*p*	OR (95% CI)
Male	45 (41.3)	24 (38.1)	21 (45.7)	0.4	1.3 (0.6-2.9)	-	-
Hospital stay, days – mean	25.4	26.8	23.5	0.558	-	-	-
ICU[5] stay, days – mean	21.2	22.6	19.1	0.578	-	-	-
Age, years – mean	53	49.9	57.1	-	-	-	-
Patient origin							
Clinical	45 (41.3)	21 (33.3)	24 (52.2)	**0.048**	**2.2 (0.9 - 4.7)**	0.1	1.9 (0.82-4.6)
Surgery	23 (21.1)	12 (19.1)	11 (23.9)	0.538	1.3 (0.5 - 3.0)	-	-
Traumatology	41 (37.6)	30 (47.6)	11 (23.9)	0.011	0.3 (0.1 - 0.8)	-	-
Comorbidity							
Heart failure	45 (41.3)	25 (39.7)	20 (43.5)	0.691	1.2 (0.5 - 2.5)	-	-
Nephropathy	17 (15.6)	9 (14.3)	8 (17.4)	0.659	1.3 (0.4 - 3.6)	-	-
Malignancy	13 (11.9)	8 (12.7)	5 (10.9)	0.771	0.8 (0.3 - 2.9)	-	-
Diabetes mellitus	19 (17.4)	11 (17.5)	8 (17.4)	0.992	0.9 (0.4 - 2.7)	-	-
Comorbidity index							
CHARLSON ≥3	26 (23.8)	15 (23.8)	11 (25.6)	0.99	1.0 (0.4 - 2.4)	-	-
Invasive procedures							
Central venous catheter	96 (88.1)	40 (87.0)	56 (88.9)	0.758	1.8 (0.3 - 2.7)	-	-
Mechanical ventilation	50 (48.9)	21 (45.6)	31 (49.2)	0.968	0.9 (0.4 - 1.8)	-	-
Vesical probe	35 (32.1)	21 (45.6)	14 (22.2)	**0.009**	**2.9 (1.2 - 6.7)**	**0.03**	**2.7 (1.1-6.9)**
Tracheostomy	70 (64.2)	28 (60.9)	42 (66.7)	0.532	0.8 (0.4 - 1.7)	-	-
Hemodialysis catheter	41 (37.6)	23 (50.0)	18 (28.6)	**0.001**	5.3 (2.1 - 12.9)	**0.01**	**3.3 (1.3-8.5)**

Table 2: Demographic, clinical and microbiological characteristics of patients with BSI caused by *Klebsiella pneumoniae* in an ICU at the Hospital of the Federal University of Uberlândia (*continued*)

Characteristics	Total *n*[1] = (%) *n* = 109 (%)	Survivors *n* (%) *n* = 63 (%)	Non-survivors *n* (%) *n* = 46 (%)	*Univariate*		*Multivariate*	
				p[2]	OR[3] (95% CI[4])	*p*	OR (95% CI)
Source							
Primary	59 (54.1)	34 (54.0)	25 (54.3)	0.96	1.1 (0.5-2.8)	-	-
Secondary	50 (45.9)	29 (46.0)	21 (45.7)	0.96	0.9 (0.4 - 2.1)	-	-
Urinary tract	18 (16.5)	9 (14.3)	9 (19.6)	0.463	1.5 (0.5 - 4.0)	-	-
Respiratory tract	32 (29.4)	20 (31.7)	12 (26.1)	0.521	0.7 (0.3 - 1.8)	-	-
Prior use of antibiotics	82 (72.2)	39 (84.8)	43 (68.2)	**0.048**	2.6 (0.9 - 6.8)	0.4	0.7 (0.3-1.7)
Inappropriate empirical therapy	68 (62.4)	35 (76.1)	33 (52.4)	**0.011**	2.9 (1.2 - 6.7)	0.8	1.2 (0.3-4.9)
Empirical use of cephalosporins	40 (36.7)	18 (39.1)	22 (34.9)	0.652	1.2 (0.5 - 2.6)	-	-
Empirical use of carbapenems	26 (23.8)	14 (30.4)	12 (19.0)	0.168	1.9 (0.7 - 4.5)	-	-
K. pneumoniae MDR[6]	28 (25.7)	15 (32.6)	13(20.6)	0.15	1.8 (0.8-4.4)	-	-
K. pneumoniae carbapenem resistant	70 (64.2)	30 (65.2)	40 (63.5)	0.852	1.1 (0.5 - 2.4)	-	-
Risk time (days-mean)	15	13.5	16.1	0.334	-	-	-
Risk time ICU (days-mean)	8.6	7.6	9.3	0.061	-	-	-

[1]*n*: number; [2]*p*: *P* value; [3]OR: Odds ratio; [4]CI: confidence interval; [5]ICU: Intense Care Unit; [6]MDR: multidrug resistant.

Table 3: Demographic, clinical and microbiological characteristics of patients with BSI caused by *Pseudomonas aeruginosa* in an ICU at the Hospital of the Federal University of Uberlândia (n = 114)

Characteristics	Total *n*[1] = (%) *n* = 114 (%)	Survivors *n* (%) *n* = 72 (%)	Non-survivors *n* (%) *n* = 42 (%)		*Univariate*		*Multivariate*
				p[2]	OR[3] (95% CI[4])	*p*	OR (95% CI)
Male sex	86 (75.4)	52 (72.2)	34 (80.9)	0.3	0.6 (0.2-1.5)	-	-
Hospital stay, days – mean	50.1	40	66.5	**0.03**	-	-	-
ICU[5] stay, days – mean	48.4	29.9	79.2	**0.01**	-	-	-
Age, years – mean	54	56.9	49.2				
Patient origin							
Clinical	46 (31.9)	32 (44.4)	14 (33.3)	0.2	1.6 (0.7-3.5)	-	-
Surgery	33 (28.9)	19 (26.4)	14 (33.3)	0.4	0.7 (0.3-1.6)	-	-
Traumatology	35 (30.7)	21 (29.2)	14 (33.3)	0.6	0.8 (0.3-1.8)	-	-
Comorbidity							
Heart failure	37 (32.5)	23 (31.9)	14 (33.3)	0.9	0.9 (0.4-2.1)	-	-
Nephropathy	23 (20.2)	16 (22.2)	7 (16.7)	0.5	1.4 (0.5-3.8)	-	-
Malignancy	24 (21.0)	19 (26.4)	5 (11.9)	0.06	2.6 (0.9-7.7)	**0.02**	**1.5 (0.3-1.1)**
Diabetes mellitus	25 (21.9)	18 (25.0)	7 (16.7)	0.2	1.7 (0.6-4.4)	-	-
Comorbidity index							
CHARLSON ≥3	42 (36.8)	30 (41.7)	12 (28.6)	0.2	1.7 (0.8-4.0)	-	-
Invasive procedures							
Central venous catheter	108 (94.7)	68 (94.4)	40 (95.2)	0.8	0.8 (0.2-4.8)	-	-
Mechanical ventilation	49 (43.0)	34 (47.2)	15 (35.7)	0.2	1.6 (0.7-3.5)	-	-
Vesical probe	101 (88.6)	63 (87.5)	38 (90.5)	0.7	0.7 (0.2-2.6)	-	-
Tracheostomy	59 (51.7)	29 (40.3)	30 (71.4)	**0.001**	**0.3 (0.1-0.6)**	0.9	0.9 (0.1-10.2)
Hemodialysis catheter	43 (37.7)	35 (48.6)	8 (19.0)	**0.001**	**4.0 (1.6-9.9)**	**0.01**	**0.2 (0.1-0.8)**

Table 3: Demographic, clinical and microbiological characteristics of patients with BSI caused by *Pseudomonas aeruginosa* in an ICU at the Hospital of the Federal University of Uberlândia (*continued*)

Characteristics	Total	Survivors	Non-survivors	*Univariate*		*Multivariate*	
	***n*[1] = (%)**	***n* (%)**	***n* (%)**				
	***n* = 114 (%)**	***n* = 72 (%)**	***n* = 42 (%)**	***p*[2]**	**OR[3] (95% CI[4])**	***p***	**OR (95% CI)**
Source							
Primary	51 (44.7)	35 (48.6)	16 (38.1)	0.27	1.54 (0.7-3.3)	-	-
Secondary	63 (55.3)	37 (51.4)	26 (61.9)	0.27	0.65 (0.2-1.4)	-	-
Urinary tract	9 (7.9)	6 (8.3)	3 (7.1)	0.8	1.2 (0.3-5.0)	-	-
Respiratory tract	50 (43.9)	28 (38.9)	22 (52.4)	0.2	0.6 (0.3-1.3)	-	-
Others	4 (3.5)	3 (4.2)	1 (2.4)	0.6	1.8 (0.2-17.7)	-	-
Prior use of antibiotics	106 (93.0)	70 (97.2)	36 (85.7)	**0.04**	**5.8 (1.1-30)**	0.07	6.5 (0.8-52.3)
Inappropriate empirical therapy	44 (38.6)	35 (48.6)	9 (21.4)	**0.04**	**3.5 (1.5-8.3)**	**0.02**	**6.5 (2.0-21.1)**
Empirical use of cephalosporins	64 (56.1)	38 (52.8)	26 (61.9)	0.3	0.7 (0.3-1.5)	-	-
Empirical use of carbapenems	66 (57.9)	47 (65.3)	19 (45.2)	**0.03**	**2.3 (1.0-4.9)**	**0.05**	**2.7 (0.9-7.9)**
Empirical use of beta lactams	15 (13.2)	11 (15.3)	4 (9.5)	0.6	1.7 (0.5-5.8)	-	-
Empirical use of polymyxins	25 (21.9)	18 (25.0)	7 (16.7)	0.3	1.7 (0.6-4.4)	-	-
P. aeruginosa MDR[6]	38 (33.3)	30 (41.7)	8 (19.0)	**0.01**	**3.0 (1.2-7.5)**	0.2	1.9 (0.6-5.8)
P. aeruginosa carbapenem resistant	54 (47.4)	36 (50.0)	18 (42.9)	0.5	1.3 (0.6-2.9)	-	-
Risk time (days-mean)	33.6	35.8	29.2	0.702	-	-	-
Risk time ICU (days-mean)	31.9	25.7	41.8	0.15	-	-	-

[1]*n*: number; [2]*p*: *P* value; [3]OR: Odds ratio; [4]CI: confidence interval; [5]ICU: Intense Care Unit; [6]MDR: multidrug resistant.

Capítulo III

A decade-long retrospective study of bloodstream infections caused by carbapenem-resistant Gram-negative bacilli in an Intensive Care Unit at a prominent referral hospital in Brazil

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ORIGINAL ARTICLE

A decade-long retrospective study of bloodstream infections caused by carbapenem-resistant Gram-negative bacilli in an Intensive Care Unit at a prominent referral hospital in Brazil.

Vinícius Lopes Dias[a], Sebastiana Sabino Silva[a], Melina Lorraine Ferreira[a], Jane Eire Uzredo Cunha[a], Iara Rossi Gonçalves[a], Bruna Fuga Araújo[b], Luiz Gustavo Machado[a], Paulo Pinto Gontijo Filho[a], Rosineide Marques Ribas[a]*.

[a]Institute of Biomedical Sciences, Laboratory of Molecular Microbiology, Federal University of Uberlândia (UFU), Uberlândia (MG), Brazil.
[b]School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil

*Corresponding author:
E-mail: rmribas@ufu.br (Ribas, RM)
Tel.: +55 34 32258574
Present Address: Laboratório de Microbiologia - Instituto de Ciências Biomédicas, Av. Amazonas, S/N, Bloco 2B, Sala 214 (Piso Superior), Campus Umuarama, CEP/Zip Code: 38400-902. Uberlândia – MG, Brazil.

Abstract

Introduction: Carbapenem-resistant Gram-negative infections, particularly in developing countries like Brazil, pose a significant concern in healthcare. This study aimed to assess the prevalence and prognostic factors of bloodstream infections (BSI) caused by carbapenem-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (KPA-GNB) over the past decade in a university hospital in Brazil. Furthermore, the study sought to evaluate the consequences of inappropriate antimicrobial therapy on patient outcomes. ***Methods***: A retrospective cohort study was conducted at a Brazilian teaching hospital to analyze patients within the ICU who had experienced BSI caused by carbapenem-resistant KPA-GNB from 2011 to 2019. ***Results***: A total of 257 patients were enrolled in this study, with 51.0% of these patients experiencing mortality. The observed rate of BSI caused by KPA-GNB was 1.98 per 1,000 patient days. Notably, during the second semester of 2014, the second semester of 2015, and from July 2017 to December 2018, the occurrence of KPA-GNB bloodstream infections exceeded the established control limit, confirming the presence

of an outbreak. The origin of these infections could not be determined in 56.4% of the cases. However, among BSI cases where the origin could be determined, the respiratory tract accounted for the highest prevalence (33.8%). The presence of malignancy, use of hemodialysis catheters, and the administration of carbapenems were identified as risk factors for mortality. **Conclusion**: The escalating prevalence and spread of carbapenem-resistant Gram-negative bacteria pose a significant threat to healthcare services, highlighting the critical role of these microorganisms as leading causes of hospital-acquired infections, emphasizing the importance of surveillance, infection control practices, and the validity of our findings supported by outbreak evidence.
Keywords: Intensive care units; Bloodstream Infections; Gram-negative bacterial infections, Multiple Drug Resistance.

Introduction

Bloodstream infections (BSI) associated with carbapenem-resistant Gram-negative bacilli (GNB) are the most frequent result of unsafe patient care worldwide, mainly in resource-limited countries, such as Brazil[1-3]. In these countries not only are the incidence and resistance of these infections significantly higher, but they are also associated with risk factors, difficulties in treatment, higher mortality, morbidity, and costs[1,4].

The data on BSI caused by carbapenem-resistant GNB in Brazil are important. However, the data accumulated from tertiary care teaching hospitals over the last ten years (2011-2019) holds valuable and meaningful research significance for understanding the epidemiology of these infections in Brazil. In this study, we conducted a comprehensive analysis of BSI caused by carbapenem-resistant GNB in an Intensive Care Unit (ICU) at a tertiary hospital in southeastern Brazil over a decade-long period (2011-2019).

This study sought to analyze the prevalence and prognostic factors of BSI caused by carbapenem-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* (KPA-GNB) over the last decade in a Brazilian university hospital. Additionally, it aimed to assess the impact of inadequate antimicrobial therapy on patient outcomes.

Methods

Patients, settings and study design

This study was carried out utilizing the database of Uberlandia University Hospital, a prominent 530-bed teaching medical facility situated in Uberlandia, Minas Gerais, Brazil. The

records were meticulously examined to pinpoint patients within the ICU who had experienced BSI caused by KPA-GNB from 2011 to 2019. Retrieval of medical data and outcomes was achieved retrospectively. For microbial identification and antimicrobial susceptibility testing, the state-of-the-art Vitek-2 system (bioMérieux, Marcy l'Etoile, France) was employed within the hospital premises.

The scope of the study encompassed exclusively nosocomial bacteremia acquired within the ICU setting. In instances where patients experienced multiple episodes of BSI, our analysis focused solely on the initial occurrence. The calculation of the endemic rate of KPA-GNB-related BSI per 1,000 patient-days was conducted following the method outlined in a previous reference.[5]

The main outcome was in ICU mortality and the measure used was a 30-day mortality rate. We also accessed secondary outcomes, including the duration of ICU stay, use of invasive procedures, and collection of GNB blood culture. For each studied patient, the following analyzed characteristics were: length of ICU stay before KPA-GNB bacteremia, length of total ICU stay, invasive procedures (central venous catheter, mechanical ventilation, vesical probe, tracheostomy, and hemodialysis catheter) and previous antibiotic use. The medical histories of the patient's underlying conditions such as heart failure, nephropathy, malignancy, and diabetes mellitus, were determined. We also accessed the search for the origin of BSI.

Definitions

Bacteremia was considered to be nosocomial if the infection occurred >48h after admission and no clinical evidence of infection on admission existed[6]. Bacteremia is classified as primary when the patient has a recognized pathogen cultured from one or more blood cultures, and the organism cultured is not associated with any infection at another site[6]. Secondary bacteremia can occur as a result of the dissemination of an infection, often originating from conditions such as pneumonia or urinary tract infections[6].

For the comparison of outcomes, survival status was evaluated at 30-days after the onset of bacteremia. Empirical therapy was defined as the administration of antibiotics before the availability of the sensitivity report. Inappropriate empirical therapy was defined as inactive antibiotic administration or delayed antibiotic therapy.

Statical analysis

The Chi-square or Fisher's exact test was used to compare discrete variables. Multivariate analysis was performed using multiple logistic regression and the values were included when significance was $p \leq 0.05$ in univariate analysis. To determine inappropriate therapy for ICU mortality within 30 days of the diagnosis of the infection, a multiple logistic regression model was used to the effects of confounding variables. All p-values ≤ 0.05 were considered statistically significant. The epidemiological data were analyzed through the programs GraphPad Prism Software 6.01 (San Diego, CA, USA) and BioEstat 5.0 (Tefé, AM, Brazil).

Results

Incidence rates

A total of 257 non-repetitive patients presetting with KPA-GNB infections in adult ICU were included in this study, over a 10-year study period, with an incidence density of 1.98 per 1,000 patient days. Regarding the endemic context, that was a substantial-fold rise in the occurrence of KPA-GNB infections from 2011 to 2014 (from 0.45 to 1.64 per 1,000 patient days). Moreover, KPA-GNB infections rates per 1,000 patients days had a progressive increase from August to December 2014, August to December 2015, and August 2017 to December 2018, when they exceed the control limit established at 3σ above the average incidence of infection, confirming a KPA-GNB outbreak. The infection acquisition rate where 2.83 per 1,000 patients days in 2nd semester of 2018 decreasing to 0.27 by 1000 patients days at the end of 2nd semester of 2019 (Figure 1).

The first outbreak, observed from August to December of 2014 had a total of 46 infections, where 37% (n=17) were caused by *K. pneumoniae* and 29.4% (n=5) of these were carbapenem-resistant. Of the 17 patients, 11 (64.7%) had previously received antibiotic therapy and had an average hospital stay of 18.8 days until the diagnosis. The second and third outbreaks had a total of 41 and 71 infections caused by KPA-GNB respectively. These two last outbreaks were characterized mainly by predominance of infections caused by *A. baumannii* which were 18 (43.9%) infections from August to December 2015 and 34 (47.9%) from August 2017 to December 2018. In terms of hospital stay until diagnosis, the first group had an average of 18 days, while the second group had an average of 23 days. Additionally,

both groups had previously received antibiotic therapy, with 15 patients in the first group and 27 patients in the second group.

The detailed information on factors associated with dead and the relevant demographic clinical characteristics of the study population are summarized in Table 1. Remarkably prolonged ICU stay, averaging approximately 41.1 days, was observed alongside significantly high mortality rates (51.0%) in 30 days. The respiratory tract was identified as the main source of these bacteremias (33.8%), while the source remained undetermined in 56.4% of cases. Empirical administration of antimicrobials was observed in 79.8% of patients, with 59.5% classified as inappropriate and/or inadequate, and carbapenems were predominantly used (38.9%).

The majority of risk factors described in the literature exhibited statistical significance in the univariate analysis, with notable highlights including the presence of diabetes mellitus (p=0.0015) and malignancy (p=0.008). Among invasive procedures, the use of vesical probe and hemodialysis catheter showed statistically significant (p=0.057 and p=<0.0001 respectively). In the multivariate analysis, the most significant risk factors were identified as follows: the presence of malignancy (p=0.0415), utilization of hemodialysis catheter (p=<0.01), and empirical use of carbapenems (p=0.025) (Table 1).

The survival distributions were depicted in Figure 2, revealing significant differences in patients with BSI caused by carbapenem-resistant non-fermenting GNB (p=0.0042) (Figure 2b), as well as in BSI caused by carbapenem-resistant KPA-GNB (p=0.0032) (Figure 2c). However, there was no significant difference in patients with BSI caused by carbapenem-resistant *K. pneumoniae* (p=0.7903) (Figure 2a).

In terms of inappropriate and appropriate therapies, there were significant differences in the 30-day survival distributions among patients with BSI caused by carbapenem-resistant *A. baumannii* and *P. aeruginosa* (p=0.0003) (Figure 2e), as well as in cases of BSI caused by carbapenem-resistant KPA-GNB (p=0.0009) (Figure 2f).

Discussion

The escalating prevalence and the spread of carbapenem-resistant Gram-negative bacteria pose a significant threat to healthcare services, where prioritized by the World Health Organization (WHO) as critical pathogens requiring urgent drug research and development[7]. These monitoring data of BSI for a large tertiary hospital Brazilian from 2011-2019

unequivocally demonstrates the critical role of these microorganisms as leading such hospital-acquired infections.

The data evidenced herein indicated the rates of infections by KPA-GNB increased approximately 6 times fold from 2011-2018 and was especially remarkably in 2014. This study clearly illustrates the presence of a preoccupancy situation regarding KPA-GNB BSI in the evaluated institution. Additionally, the analysis hospital database revealed outbreaks with the prevalence of carbapenem-resistant *K. pneumoniae* detected in 2nd semester of 2014, *A. baumannii* in 2nd semester of 2015, and from July 2017 to December 2018. Consistent with previous reports these outbreaks were primarily associated with extended hospital stays with a significant number of patients receiving previous therapy with antibiotics. Given the circumstances, the hospital has proactively implemented an infection control service to mitigate the transmission of resistant strains and advocate for the prudent utilization of antibiotics. Our data further emphasize the critical importance of comprehensive surveillance and infection control practices.

Over the past decade, a multitude of studies have consistently underscored the well-recognized correlation between mortality risk in patients with severe infections, specifically bloodstream infections caused by carbapenem-resistant KPA-GNB, and the selection of empirical antimicrobial therapy. In this context, our study reinforces the perspective that the heightened probability of inappropriate empirical antibiotic therapy and the empirical usage of carbapenems, linked to the resistant phenotype of the assessed microorganisms, appears to contribute to a poor prognosis for patients afflicted by these infections.

Numerous studies conducted in the region and Brazil have reported multiple outbreaks caused by these three microorganisms, as documented in numerous studies[9-12]. The presence of these outbreaks supports the validity and relevance of our data, providing further evidence that our findings accurately reflect the current reality.

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Ethical statement

This study was approved by the Committee of the Federal University of Uberlandia (228/11).

Conflict of Interests

The authors have no conflict of interest to declare.

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Legends of figures:

Figure 1. Endemic level of Bloodstream infections (BSI) caused by *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter baumannii* (KPA-GNB) per 1,000 patient-days from January 2011 to December 2019. Upper control limit (3σ + X); upper alert limit (2σ + X);

Figure 2. Kaplan-Meier curve for survival of BSI caused by carbapenem resistant GNB in an ICU at the Hospital of the Federal University of Uberlândia. **a**. Curve for survival for BSI caused by carbapenem resistant *K. pneumoniae* (p=0.7903); **b**. Curve for survival for BSI caused by carbapenem resistant *A. baumannii* and *P. aeruginosa* (p=0.0042); **c**. Curve for survival for BSI caused by carbapenem resistant KPA-GNB (p=0.0032); **d**. Curve for survival of inappropriate and appropriate therapies for BSI caused by carbapenem resistant *K. pneumoniae* (p=0.8286); **e**. Curve for survival of inappropriate and appropriate therapies for BSI caused by carbapenem resistant *A. baumannii* and *P. aeruginosa* (p=0.003); **f**. Curve for survival of inappropriate and appropriate therapies for BSI caused by carbapenem resistant KPA-GNB (p=0.009).

Non-standard Abbreviations

GNB: Gram-negative bacilli

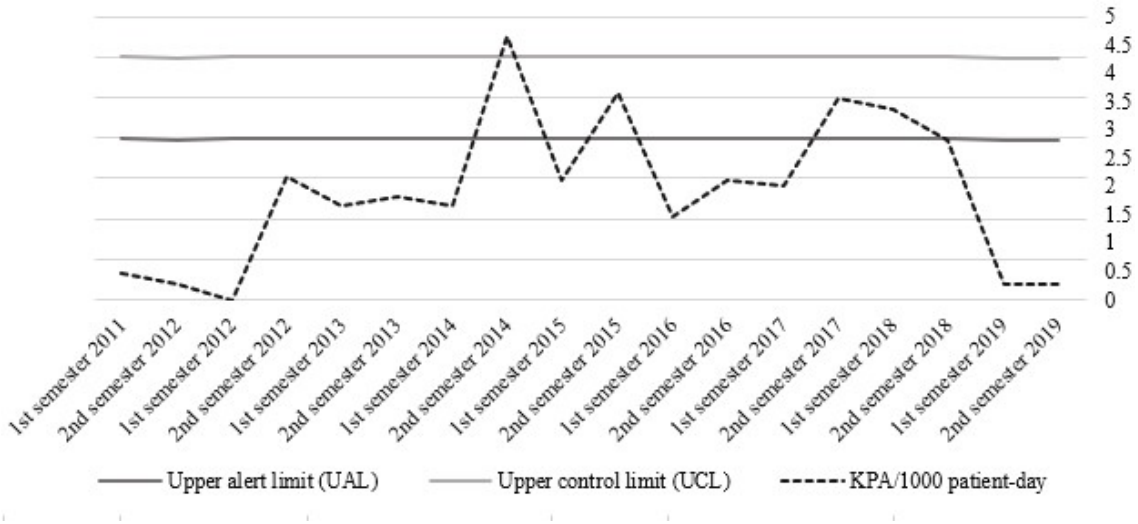
ICU: Intensive Care Unit

KPA-GNB*: K. pneumoniae, P. aeruginosa* and *A. baumannii*

UTIs: Urinary tract infections

WHO: World Health Organization

Figure 1.


5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1st semester 2011
2nd semester 2012
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1st semester 2015
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1st semester 2016
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1st semester 2017
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2nd semester 2018
1st semester 2019
2nd semester 2019
Upper alert limit (UAL)
Upper control limit (UCL)
KPA/1000 patient-day

Figure 2.

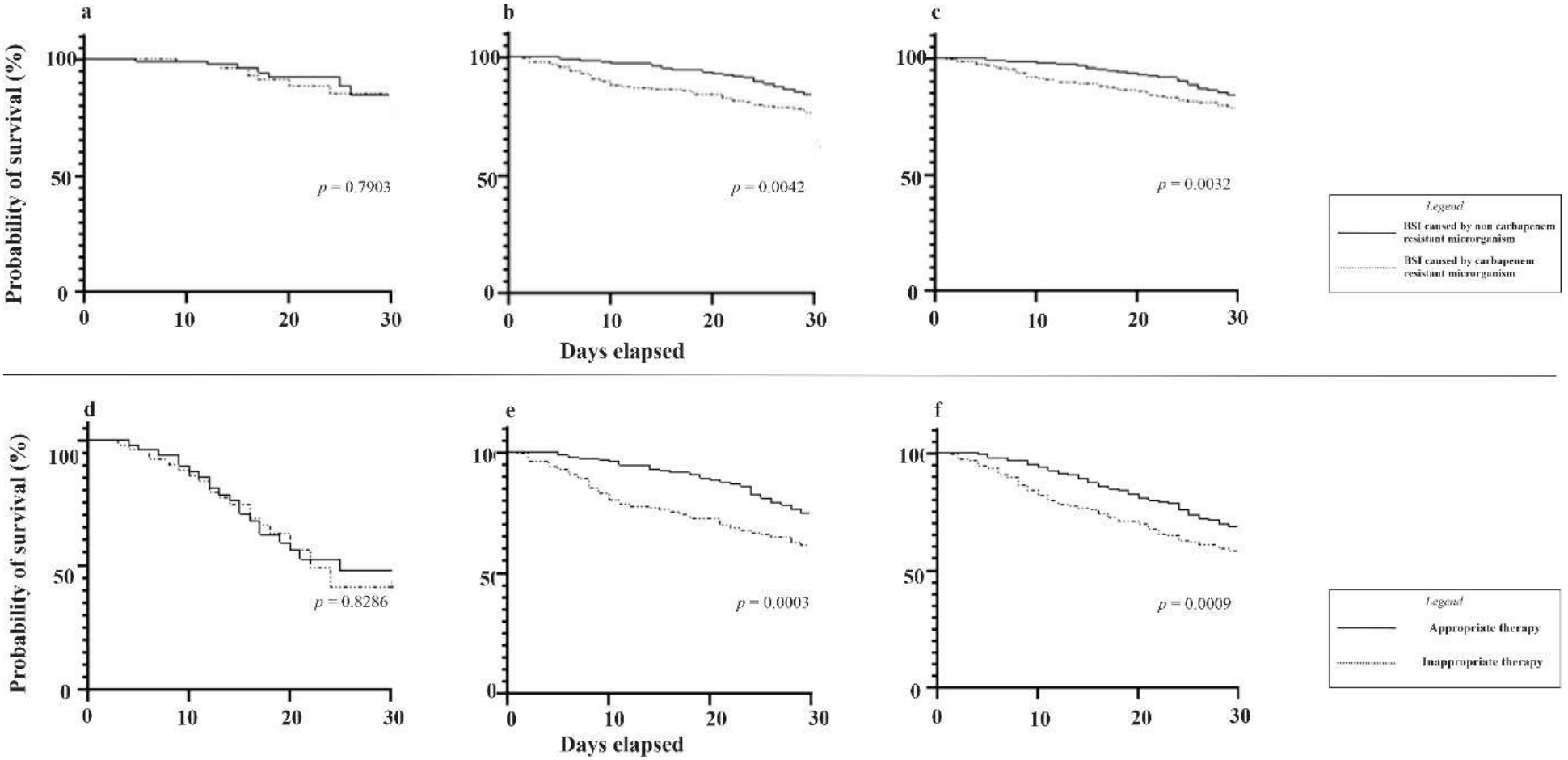
a
b
c
d
e
f
Probability of survival (%)
100
50
0
0
10
20
30
Days elapsed
p = 0.7903
p = 0.0042
p = 0.0032
p = 0.8286
p = 0.0003
p = 0.0009
Legend
BSI caused by non carbapenem resistant microrganism
BSI caused by carbapenem resistant microrganism
Legend
Appropriate therapy
Inappropriate therapy

Table 1– Characteristics of patients presenting bloodstream infections (BSI) caused by *K. pneumoniae, P. aeruginosa* and *A. baumannii* (KPA-GNB) carbapenem resistant admitted to the Intense Care Unit (ICU) of the Clinical Hospital belonging to the Federal University of Uberlândia, Brazil, between January 2011 and December 2019.

Characteristics	***n*[1] = (%)** ***n* = 257 (%)**	**Non-Survivors** ***n* = (%)** ***n* = 131 (%)**	**Survivors** ***n* = (%)** ***n* = 126 (%)**	***Univariate***		***Multivariate***	
				***p*[2]**	**OR[3] (95% CI[4])**	***p***	**OR (95% CI)**
Male	168 (65.4)	86 (65.6)	82 (65.1)	0.9236	0.97 (0.58 - 1.64)	-	-
Age, years – mean	52.55	58.05	45.3	-	-	-	-
Origin of patient							
Clinical	114 (44.4)	78 (59.5)	36 (28.6)	**<0.0001**	0.27 (0.16 - 0.45)	0.059	2.05 (0.97 - 4.33)
Surgery	58 (22.6)	28 (21.4)	30 (23.8)	0.6406	1.15 (0.64 - 2.08)	-	-
Traumatology	85 (33.0)	26 (19.9)	59 (46.8)	**<0.0001**	3.56 (2.01 - 6.17)	0.5457	0.79 (0.37 - 1.69)
Comorbidities							
Heart failure	120 (46.7)	64 (48.8)	56 (44.4)	0.4768	0.84 (0.52 - 1.38)	-	-
Nephropathy	37 (14.4)	22 (16.8)	15 (11.9)	0.2644	0.67 (0.33 - 1.35)	-	-
Malignancy	43 (16.7)	32 (24.4)	11 (8.70)	**0.0008**	0.30 (0.14 - 0.63)	**0.0415**	3.13 (1.04 - 9.38)
Diabetes mellitus	39 (15.2)	29 (22.1)	10 (7.90)	**0.0015**	0.30 (0.15 - 0.64)	0.1703	1.91 (0.76 - 4.83)
Comorbidity index							
CHARLSON ≥3	78 (35.4)	52 (39.7)	26 (20.6)	**0.0009**	0.39 (0.23 - 0.68)	0.7322	0.85 (0.34 - 2.12)
Invasive procedures							
Central venous catheter	238 (92.6)	129 (98.5)	109 (86.5)	0.0655	0.14 (0.01 - 0.86)	-	-
Mechanical ventilation	68 (26.5)	40 (30.5)	28 (22.2)	0.1311	0.65 (0.37 - 1.14)	-	-
Vesical probe	225 (87.5)	122 (93.1)	103 (81.7)	**0.0057**	0.34 (0.15 - 0.76)	0.2888	0.54 (0.17 - 1.69)
Tracheostomy	144 (56.0)	68 (51.9)	76 (60.3)	0.1745	1.41 (0.85 - 2.28)	-	-
Hemodialysis catheter	93 (36.2)	71 (54.2)	22 (17.5)	**<0.0001**	0.18 (0.10 - 0.31)	**<0.001**	3.59 (1.93 - 1.66)

Table 1– Characteristics of patients presenting bloodstream infections (BSI) caused by *K. pneumoniae, P. aeruginosa* and *A. baumannii* (KPA-GNB) carbapenem resistant admitted to the Intense Care Unit (ICU) of the Clinical Hospital belonging to the Federal University of Uberlândia, Brazil, between January 2011 and December 2019 (*continued*).

Characteristics	***n*[1] = (%)** **n = 257 (%)**	**Non-Survivors** ***n* = (%)** **n = 131 (%)**	**Survivors** ***n* = (%)** **n = 126 (%)**	***Univariate***		***Multivariate***	
				***p*[2]**	**OR[3] (95% CI[4])**	***p***	**OR (95% CI)**
Source							
Primary	145 (56.4)	69 (52.7)	76 (60.3)	0.2166	1.37 (0.82 - 2.21)	-	-
Secondary	112 (43.6)	62 (47.3)	50 (39.7)	0.2166	0.73 (0.45 - 1.22)	-	-
Urinary tract	17 (6.6)	9 (6.90)	8 (6.30)	0.8666	0.92 (0.35 - 2.30)	-	-
Respiratory tract	87 (33.8)	46 (35.1)	41 (32.5)	0.6628	0.89 (0.53 - 1.50)	-	-
Others	8 (3.1)	7 (5.30)	1 (0.80)	0.93	1.97 (0.32 - 2.91)	-	-
Use of antibiotics							
Prior use of antibiotics	205 (79.8)	112 (85.5)	93 (73.8)	**0.0197**	2.09 (1.10 - 3.83)	0.5213	1.28 (0.60 - 2.77)
Inappropriate empirical therapy	153 (59.5)	85 (64.9)	68 (54.0)	0.0747	1.58 (0.97 - 2.59)	-	-
Empirical use of carbapenems	100 (38.9)	67 (51.1)	33 (26.2)	**<0.0001**	2.95 (1.75 - 4.97)	**0.025**	2.07 (1.10 - 3.93)

[1]*n*: number; [2]*p*: *P* value; [3]OR: Odds ratio; [4]CI: confidence interval.

Capítulo IV

Exploring Virulence Genes, Carbapenemase-Encoding Genes, and Clonality in Carbapenem-Resistant *Klebsiella pneumoniae* Isolates: Insights from Public Hospitals in the Southeast Region of Brazil

Artigo preparado para submissão e consequente publicação no periódico Journal of Medical Microbiology em 2024.

ORIGINAL ARTICLE

Exploring Virulence Genes, Carbapenemase-Encoding Genes, and Clonality in Carbapenem-Resistant *Klebsiella pneumoniae* Isolates: Insights from Public Hospitals in the Southeast Region of Brazil

Vinícius Lopes Dias[a], Paola Amarel de Campos[a], Iara Rossi Gonçalves[a], Vitelhe Ferreira de Almeida[a], Luiz Gustavo Machado[a], Paulo Pinto Gontijo Filho[a], Rosineide Marques Ribas[a]*.

[a]Institute of Biomedical Sciences, Laboratory of Molecular Microbiology, Universidade Federal de Uberlândia (UFU), Uberlândia (MG), Brazil.

*Corresponding author:
E-mail: rmribas@ufu.br (Ribas, RM)
Tel.: +55 34 32258574
Present Address: Laboratório de Microbiologia - Instituto de Ciências Biomédicas, Av. Amazonas, S/N, Bloco 2B, Sala 214 (Piso Superior), Campus Umuarama, CEP/Zip Code: 38400-902. Uberlândia – MG, Brazil.

ABSTRACT

Introduction: The widespread dissemination of carbapenem-resistant *Klebsiella pneumoniae*, particularly in developing nations like Brazil, poses a significant healthcare challenge. This study aimed to examine the presence of virulence genes and carbapenemase-encoding genes, as well as determine the clonality of carbapenem-resistant *K. pneumoniae* isolates obtained from public hospitals in the Southeast region of Brazil. ***Methods***: A prospective study was conducted by collecting carbapenem-resistant *K. pneumoniae* strains from multiple hospitals in the region. The investigation of virulence genes, resistance genes, and the clonal dissemination of selected strains colistin-resistant was carried out using Polymerase Chain Reaction (PCR) and pulsed-field gel electrophoresis (PFGE) techniques. ***Results***: Overall, out of the 165 carbapenem-resistant *K. pneumoniae* strains analyzed, 47.9% (79), 10.9% (18), and 72.7% (120) were found to harbor the *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ genes, respectively. Notably, 29.7% (49) of these isolates exhibited resistance to polymyxin. PCR analysis revealed the presence of virulence-associated genes, with the *entb, kfu, ureA, wabG*, and *allS* genes detected in 91.5% (151), 89.7% (148), 94.5% (156), 97.6% (161), and 11.5% (19) of the isolates, respectively. Within the subset of 49 strains resistant to polymyxin, approximately 12.2% carried the *allS* gene, while 91.8% coharbored the *entb, kfu, ureA*, and *wabG* genes. The analysis of genetic similarity using PFGE revealed a total of five patterns

(A-E) among the 15 isolates analyzed, with the B pulsotype pattern being the most predominant, followed by pulsotype A. ***Conclusion***: Infections caused by multidrug-resistant *K. pneumoniae* represent a significant challenge in terms of infection control and treatment for both ICU and non-ICU patients, as evidenced by the findings of this study. The transmission characteristics and mechanisms of resistance and virulence associated with this pathogen warrant further attention and investigation.

Keywords: Carbapenem-Resistant Enterobacteriaceae, *Klebsiella pneumoniae*, Multiple Drug Resistance, Virulence Factors

Introduction

The global spread of multi-resistant *Klebsiella pneumoniae* represents a potential clinical threat with devastating effects on patient outcomes[1]. In 2017, the World Health Organization (WHO) designated carbapenem-resistant Enterobacteriaceae (CRE) as the highest priority category[2]. In bloodstream infections, *K. pneumoniae* ranked the third position in bloodstream infections most prevalent isolates, and the first in the GNB[3]. The resistance due to carbapenem is worrisome and the major mechanisms underlying in theses strains is associated with carbapenemase production, with carbapenemase producing *K. pneumoniae* such as KPC (*K. pneumoniae* carbapenemase) or NDM-1 (New Delhi metallo-beta-lactamase) are commonly reported[4-6].

The carbapenem-resistant *K. pneumoniae* (CRKP) strains carrying the bla_{KPC} and bla_{NDM} genes consistently coharbor multiple other types of resistance genes, including extended-spectrum beta-lactamase (ESBL) genes, fluoroquinolone resistance genes, and aminoglycoside resistance genes and virulence[7,8]. This accumulation of resistance genes inevitably leads to a phenotypic multidrug-resistant (MDR) profile[8].

In order to determine the prevalence of carbapenemase-encoding genes and virulence genes, we examined isolates collected from Public Hospitals in the Southeast region of Brazil. Additionally, we employed pulsed-field gel electrophoresis (PFGE) to assess the clonality of these strains.

METHODS

Carbapenem resistant strains data collection

We conducted a prospective study in public hospitals in the city of Uberlândia (Minas Gerais, Brazil). Carbapenem-resistant *K. pneumoniae* isolates were systematically collected from clinical specimens in multiple hospitals within the city between January and December 2021. The isolates were selected based on their primary resistance to carbapenems.

Maldi-TOF/TOF, Autoflex III Smartbeam (Bruker Daltonics) and the Vitek-2 compact system (bioMérieux, France) were used for identification and antimicrobial susceptibility testing. The isolates were stored in Brain Heart Infusion (BHI) broth with glycerol at -20 °C.

Extraction of DNA and polymerase chain reaction (PCR)

Total DNA was isolated using the QIAamp DNA Micro Kit (QIAGEN, Germany). The extracted DNA was quantified and stored at -20 °C. PCR was conducted to detect the resistance and virulence genes, in each sample containing 12.5 µL of PCR mix (Taq DNA Polymerase Master Mix Red, Ampliqon, Denmark), 10.2 µL of ultrapure water, 1 µL (10 ng) of total DNA and 0.65 µL of each primer in a final volume of 25 µL.

Target genes, primers used and specific annealing temperature of PCR are given in Table 1. After 5 min at 95°C, there were 30 cycles of 95°C, 1 min.; annealing temperature, 1 min.; and 72°C, 1 min. followed by a final elongation of 5 min at 72°C. Amplified products were separated using 2% agarose and observed under ultraviolet light.

Pulsed-field gel eletrophoresis (PFGE)

Fifteen select isolates of carbapenem-resistant K. pneumoniae, chosen as representatives from the study, were subjected to typing. This process followed the PulseNet One-Day (24–28 hour) Standardized Laboratory Protocol, utilizing the XbaI enzyme and employing the PFGE technique[9].

Band patterns were analyzed through visual comparison between samples and through the GelAnalyzer 19.1 using the Dice similarity coefficient and the Unweighted Pair Group Method Using Arithmetic Averages (UPGMA) for cluster analysis. and construction of dendrograms.

RESULTS

Overall, from 165 carbapenem-resistant *K. pneumoniae* strains analyzed, 47.9% (79), 10.9% (18), and 72.7% (120) harbored the *bla*$_{KPC}$, *bla*$_{NDM}$ and *bla*$_{VIM}$ genes respectively. Notably, 29.7% (49) of these isolates exhibited resistance to polymyxin. Additionally, 7.9% (13) of the strains coharbored *bla*$_{NDM}$ and *bla*$_{VIM}$, while 35.7% (59) coharbored *bla*$_{KPC}$, *bla*$_{TEM}$ and *bla*$_{VIM}$ (Figure 1). PCR analysis revealed that the virulence-associated genes *entb*, *kfu*, *ureA*, *wabG*, and *allS* were detected in 91.5% (151), 89.7% (148), 94.5% (156), 97.6% (161), and 11.5% (19) of the isolates, respectively. Among these, 10.8% coharbored *entb*, *ureA*, *wabG*, and *allS* (Figure 1). Within the subset of 49 strains resistant to polymyxin, approximately 12.2% carried the *allS* gene, while 91.8% coharbored the *entb*, *kfu*, *ureA*, and *wabG* genes (Figure 2).

Fifteen strains were randomly selected for analysis using the PFGE technique. Among these, ten were infection samples and five were colonization samples. Among these fifteen strains, seven exhibited resistance to polymyxins. The analysis of genetic similarity revealed a total of five PFGE patterns (A-E) among the fifteen isolates analyzed, with a predominance of the B pulsotype pattern (six), followed by pulsotype A (four) (Figure 3).

DISCUSSION

Carbapenem-resistant *K. pneumoniae*, present a profound and concerning threat, as they can lead to mortality rates as alarmingly high as 40 to 50%[10]. Regrettably, the emergence of resistance to "last-line" drugs, such as colistin, is becoming increasingly prevalent in carbapenem-resistant *K. pneumoniae* strains[11,12]. Moreover, in certain instances, these isolates demonstrate resistance to all tested antibiotics, which exacerbates the gravity of the situation[13].

Our data confirmed the dissemination of the *bla*$_{KPC}$ gene and other important genes such as *bla*$_{NDM}$, *bla*$_{TEM}$ and *bla*$_{VIM}$. It was possible to observe that some strains included in the study had concomitantly one or more carbapenemase coding gene, already shows a bad epidemiological indicator. Furthermore, this study demonstrates a significant presence of virulence genes (*entB, kfu, ureA, wabG*) in these strains, highlighting the extensive range of virulence factors (capsule, lipopolysaccharide, fimbriae, and siderophores) possessed by these isolates. Recent data suggest a relationship between *K. pneumoniae* virulence and drug resistance[14].

Among the samples analyzed for clonality by PFGE, a significant majority of isolates displayed identical profiles in both clinical and rectal cultures. This compelling data serves as conclusive evidence, firmly supporting the notion that the transmission of carbapenem-resistant *K. pneumoniae* within Uberlândia Hospitals is primarily facilitated through interactions between healthcare professionals and patients. Moreover, these findings reinforce the idea that this dissemination could occur both within and beyond the hospital setting, highlighting the potential for both intra-hospital and extra-hospital transmission.

Several studies provide compelling evidence supporting the intrahospital transmission of KPC, particularly in relation to *K. pneumoniae*. These studies indicate that such transmission primarily takes place within the hospital environment, and it involves mechanisms of polyclonal propagation and the dissemination of highly successful clones[15-18].

The findings this study have clearly highlighted the role of reservoirs in the transmission of carbapenem-resistant *K. pneumoniae*. These findings underscore the urgent requirement for effective control strategies and preventive measures to combat infections. Moreover, promoting improved hand hygiene practices among healthcare workers is essential in minimizing pathogen transmission. Additionally, our data supports the need to implement restrictions on antibiotic use as a crucial approach in combating the emergence and spread of carbapenem-resistant *K. pneumoniae*.

In conclusion, our data unequivocally emphasizes the critical importance of implementing, maintaining, and strengthening comprehensive surveillance programs for Healthcare-Associated Infections caused by carbapenem-resistant *K. pneumoniae*. Moreover, it is of utmost importance to enhance and improve hospital containment measures in order to effectively mitigate the spread of resistance associated with successful clones, not only within individual hospitals but also across multiple healthcare facilities.

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Ethical statement

This study was approved by the Committee of the Federal University of Uberlandia (228/11).

Conflict of Interests

The authors have no conflict of interest to declare.

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Legends of figures:

Figure 1. Venn Diagram of the distribution of resistance and virulence genes in clinical strains of carbapenem-resistant *Klebsiela pneumoniae* detected by the PCR technique in the city of Uberlândia in the year 2021.

Figure 2. Heat map of the resistante and virulence genes of *Klebsiella pneumoniae* resistant to polymixin detected by the PCR technique in the city of Uberlândia in the year 2021.

Figure 3. Dendrogram generated by computerized analysis of clonal DNA profiles of 15 clinical samples of carbapenem-resistant *Klebsiella pneumoniae* based on pulsed-field gel electrophoresis (PFGE). The analysis was performed using the Dice/UPGMA method (similarity ≥ 80%).

Non-standard Abbreviations

GNB: Gram-negative bacilli

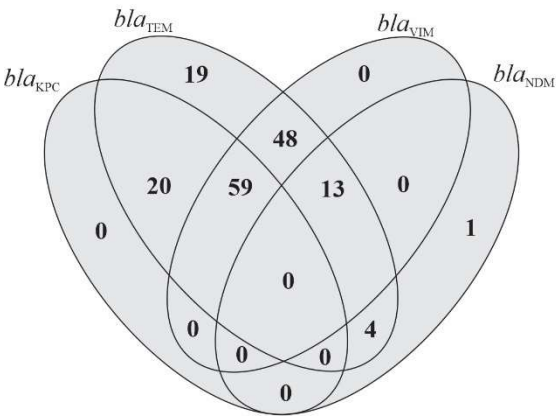
ICU: Intensive Care Unit

KPA-GNB*: K. pneumoniae, P. aeruginosa* and *A. baumannii*

UTIs: Urinary tract infections

WHO: World Health Organization

Figure 1.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bla_{VIM}
bla_{KPC}
bla_{NDM}
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20
59
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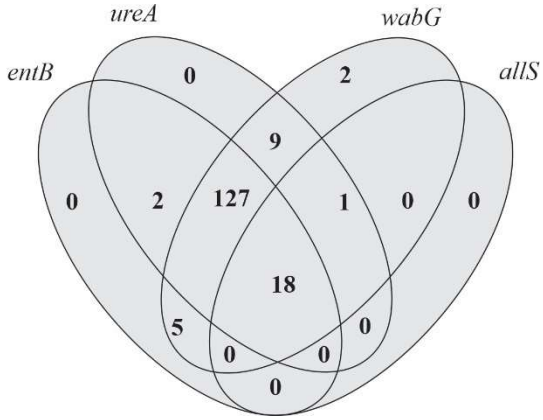
0
0
1
0
0
4
0
0
0
0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wabG
entB
allS
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1
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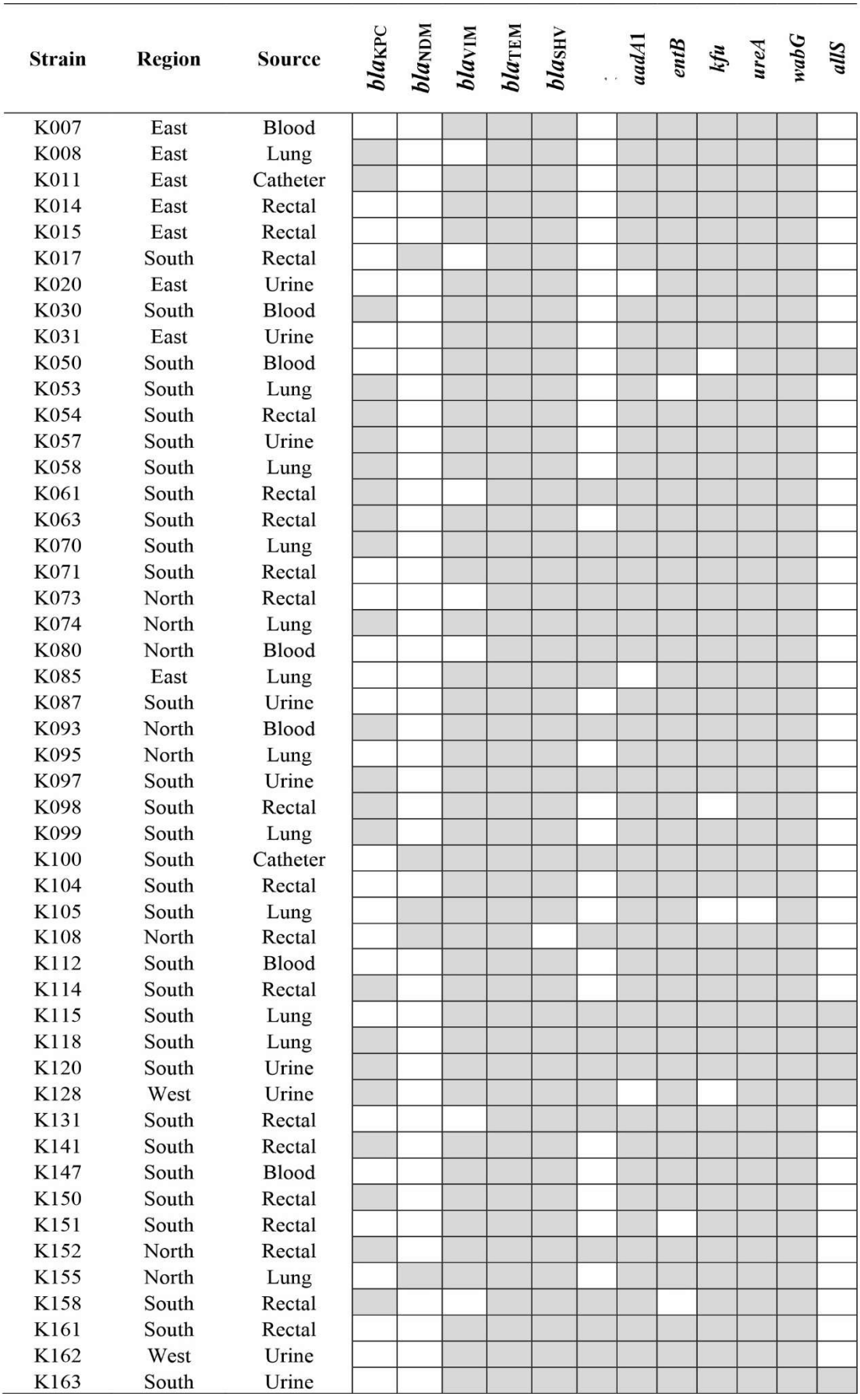
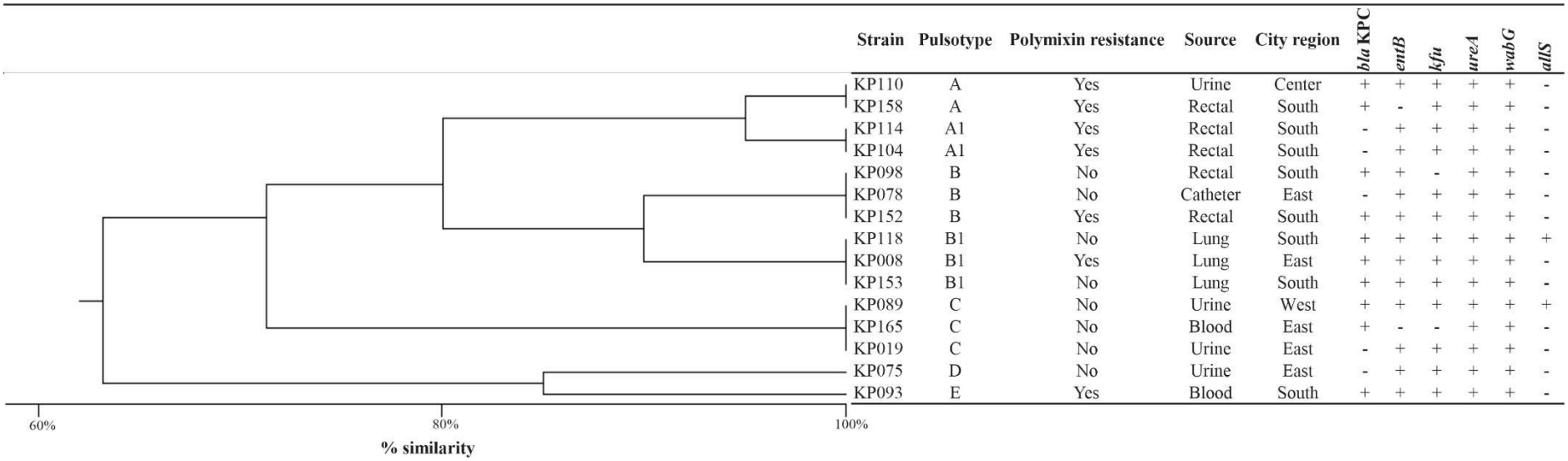
Figure 2.



Strain	Region	Source	*bla*KPC	*bla*NDM	*bla*VIM	*bla*TEM	*bla*SHV	. .	*aadA1*	*entB*	*kfu*	*ureA*	*wabG*	*allS*
K007	East	Blood												
K008	East	Lung												
K011	East	Catheter												
K014	East	Rectal												
K015	East	Rectal												
K017	South	Rectal												
K020	East	Urine												
K030	South	Blood												
K031	East	Urine												
K050	South	Blood												
K053	South	Lung												
K054	South	Rectal												
K057	South	Urine												
K058	South	Lung												
K061	South	Rectal												
K063	South	Rectal												
K070	South	Lung												
K071	South	Rectal												
K073	North	Rectal												
K074	North	Lung												
K080	North	Blood												
K085	East	Lung												
K087	South	Urine												
K093	North	Blood												
K095	North	Lung												
K097	South	Urine												
K098	South	Rectal												
K099	South	Lung												
K100	South	Catheter												
K104	South	Rectal												
K105	South	Lung												
K108	North	Rectal												
K112	South	Blood												
K114	South	Rectal												
K115	South	Lung												
K118	South	Lung												
K120	South	Urine												
K128	West	Urine												
K131	South	Rectal												
K141	South	Rectal												
K147	South	Blood												
K150	South	Rectal												
K151	South	Rectal												
K152	North	Rectal												
K155	North	Lung												
K158	South	Rectal												
K161	South	Rectal												
K162	West	Urine												
K163	South	Urine												

Figure 3.



Strain	Pulsotype	Polymixin resistance	Source	City region	*bla* KPC	*entB*	*kfu*	*ureA*	*wabG*	*allS*
KP110	A	Yes	Urine	Center	+	+	+	+	+	-
KP158	A	Yes	Rectal	South	+	-	+	+	+	-
KP114	A1	Yes	Rectal	South	-	+	+	+	+	-
KP104	A1	Yes	Rectal	South	-	+	+	+	+	-
KP098	B	No	Rectal	South	+	+	-	+	+	-
KP078	B	No	Catheter	East	-	+	+	+	+	-
KP152	B	Yes	Rectal	South	+	+	+	+	+	-
KP118	B1	No	Lung	South	+	+	+	+	+	+
KP008	B1	Yes	Lung	East	+	+	+	+	+	-
KP153	B1	No	Lung	South	+	+	+	+	+	-
KP089	C	No	Urine	West	+	+	+	+	+	+
KP165	C	No	Blood	East	+	-	-	+	+	-
KP019	C	No	Urine	East	-	+	+	+	+	-
KP075	D	No	Urine	East	-	+	+	+	+	-
KP093	E	Yes	Blood	South	+	+	+	+	+	-

Table 1. Oligonucleotides used in this study.

Target gene	Sequence (5'– 3')	Product Size (bp)	Annealing temperature	Reference
bla$_{KPC}$	CGTCTAGTTCTGCTGTCTTG CTTGTCATCCTTGTTAGGCG	798	55ºC	[19]
bla$_{NDM}$	GGTTTGGCGATCTGGTTTTC CGGAATGGCTCATCACGATC	621	55ºC	[19]
bla$_{VIM}$	GATGGTGTTTGGTCGCATA CGAATGCGCAGCACCAG	390	55ºC	[19]
bla$_{TEM}$	ATGAGTATTCAACAT TTCCG CTGACAGTTACCAATGCTTA	858	50ºC	[20]
bla$_{SHV}$	TTTATCGGCCYTCACTCAAGG GCTGCGGGCCGGATAACG	896	58ºC	[20]
qnrA	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	627	62ºC	[21]
*aadA*1	ATGAGGGAAGCGGTGATCG TTATTTGCCGACTACCTTGGTG	792	62ºC	[22]
mcr-1	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	309	58ºC	[23]
entB	GTCAACTGGGCCTTTGAGCCGTC TATGGGCGTAAACGCCGGTGAT	400	60ºC	[24]
kfu	GGCCTTTGTCCAGAGCTACG GGGTCTGGCGCAGAGTATGC	638	60ºC	[24]
allS	CATTACGCACCTTTGTCAGC GAATGTGTCGGCGATCAGCTT	764	60ºC	[24]
wabG	CGGACTGGCAGATCCATATC ACCATCGGCCATTTGATAGA	683	46ºC	[25]
ureA	GCTGACTTAAGAGAACGTTATG GATCATGGCGCTACCT(C/T)A	337	46ºC	[25]

Capítulo IV

Considerações Finais

Os resultados desse estudo permitem melhor compreender a epidemiologia de infecções causadas pelos principais bacilos Gram-negativos em pacientes da UTI no Hospital de Clínicas da Universidade Federal nos últimos dez anos. Embora a vigilância epidemiológica tenha sido realizada em apenas um centro médico, o período de dez anos proporcionou um panorama dessas infecções, bem como a crescente taxa das mesmas causadas por microrganismos resistentes. Além disso, foi possível entender como está a disseminação de algumas de linhagens clínicas de *K. pneumoniae* resistentes aos carbapenêmicos de hospitais da referida cidade.

Adicionalmente, os resultados obtidos permitiram as seguintes considerações:

- O estudo revelou uma alta frequência de isolados multirresistentes e resistentes a carbapenêmicos em infecções sanguíneas causadas por *A. baumannii, K. pneumoniae* e *P. aeruginosa*, associados a um prognóstico desfavorável em 47,0% dos pacientes. Além disso, constatou-se que em 58,8% dos casos de antibioticoterapia empírica inapropriada, os fármacos não eram eficazes contra os microrganismos presentes. Fatores de risco como o uso de traqueostomia, cateter de hemodiálise e terapia empírica inadequada também foram identificados. Em conclusão, infecções causadas por *A. baumannii, K. pneumoniae* e *P. aeruginosa* multirresistentes estão associadas a um prognóstico desfavorável em comparação com as infecções por microrganismos não multirresistentes.
- Os dados apresentados também revelam um aumento significativo, aproximadamente seis vezes, nas taxas de infecções por *A. baumannii, K. pneumoniae* e *P. aeruginosa* entre 2011 e 2018, sendo especialmente preocupante o ano de 2014. Este estudo destaca a gravidade da situação em relação às infecções sanguíneas causadas por *A. baumannii, K. pneumoniae* e *P. aeruginosa* na UTI de adultos na Instituição avaliada.
- Nossos resultados evidenciaram a disseminação do gene bla_{KPC}, bem como outros genes relevantes, como bla_{NDM}, bla_{TEM} e bla_{VIM}. Foi observado que alguns isolados analisados apresentavam múltiplos genes codificadores de carbapenemases, o que representa um indicador epidemiológico preocupante. Além disso, este estudo revela uma presença significativa de genes de virulência, como *entB*, *kfu*, *ureA* e *wabG*, indicando uma ampla variedade de fatores de virulência associados.

- A análise de clonalidade por PFGE revelou que a maioria dos isolados apresentava perfis idênticos em culturas clínicas e retais, fornecendo evidências convincentes que sustentam a transmissão de *K. pneumoniae* resistente aos carbapenêmicos dentro dos Hospitais de Uberlândia. Esses resultados indicam que as interações entre profissionais de saúde e pacientes desempenham um papel fundamental na facilitação dessa transmissão. Essas descobertas fornecem uma base conclusiva para a compreensão da dinâmica da disseminação dessa cepa resistente no ambiente hospitalar.

A capacidade dos bacilos Gram-negativos não fermentadores, bem como *K. pneumoniae* em causar infecções e persistir em ambientes hospitalares, contribui para sua rápida expansão. Os aspectos discutidos neste estudo são essenciais para o desenvolvimento de futuras estratégias de controle mais eficazes, visando conter a disseminação de *K. pneumoniae* bem como outros microrganismos e combater a resistência antimicrobiana. O conhecimento adquirido é fundamental para avançar na abordagem desses patógenos bem-sucedidos, melhorando assim a segurança e o cuidado dos pacientes.