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RESISTÊNCIA BACTERIANA E POTENCIAL TERAPÊUTICO: CARBAPENEMASES E O USO DE EXTRATOS DE PLANTAS MEDICINAIS NA TRÍPLICE FRONTEIRA (BRASIL, PARAGUAI E ARGENTINA)

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Orientação: Dra. Daniela Dib Gonçalves

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RESISTÊNCIA BACTERIANA E POTENCIAL TERAPÊUTICO:
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ALINE CRISTIANE CECHINEL ASSING BATISTA

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(Mateus 6:33)

BATISTA, Aline Cristiane Cechinel. **Resistência bacteriana e potencial terapêutico:** Carbapenemases e o uso de extratos de plantas medicinais na tríplice fronteira (Brasil, Paraguai e Argentina). Orientadora: Daniela Dib Gonçalves. Ano. 2025. 125 f. Tese (Doutorado em Ciência Animal com Ênfase em Produtos Bioativos) - Universidade Paranaense, Umuarama, 2025.

RESUMO

A resistência bacteriana representa uma das maiores ameaças à saúde pública global, ainda mais quando agravada pela disseminação de cepas resistentes aos carbapenêmicos, como *Klebsiella pneumoniae* produtora de carbapenemases (KPC e NDM). Diante disso, a tríplice fronteira entre Brasil, Paraguai e Argentina, marcada por intensa mobilidade populacional, caracteriza-se como uma região de alto risco para o desenvolvimento de surtos epidêmicos relacionados a essas cepas com perfil de multirresistência. Dessa forma, esse trabalho teve por objetivo investigar o perfil de resistência de *Klebsiella pneumoniae* produtora de carbapenemases (KPC e NDM) em um hospital de urgência na tríplice fronteira entre Brasil, Paraguai e Argentina, compreendendo os aspectos sociodemográficos dos pacientes afetados e avaliar a eficácia dos extratos de *Terminalia catappa, Banisteriopsis caapi* e *Psychotria viridis* contra essas cepas resistentes. Analisou-se 48 casos de *K. pneumoniae* KPC e/ou NDM, com amostras coletadas principalmente em UTIs. Observou-se resistência de 100% às penicilinas, cefalosporinas e carbapenêmicos; 95,8% aos aminoglicosídeos e sulfonamidas; e menor resistência à colistina (22,9%) e ceftazidima/tazobactam (39,6%). As cepas de KPC (60,4%) foram mais prevalentes, seguidas pelas de NDM (27,1%) e pela co-resistência (12,5%). O perfil clínico dos pacientes atingiu mais homens, com idade média de 64 anos, residentes em Foz do Iguaçu e usuários do sistema público de saúde. Acerca do potencial terapêutico, os extratos de *Terminalia catappa* (7 copas), *Banisteropsis caapi* e *Psychotria viridis* (*Ayahuasca*) foram testados quanto à atividade antimicrobiana, através da técnica de Microdiluição em caldo frente a algumas cepas de *K. pneumoniae* selecionadas (10 cepas KPC, 10 cepas NDM e 5 cepas co-resistentes). *P. viridis* demonstrou a melhor eficácia, com concentração inibitória mínima (CIM) entre 2,5 mg/mL e 5 mg/mL, enquanto *B. caapi* apresentou resultados muito próximos. Já *T. catappa* apresentou uma CIM de 10 mg/mL. Em contrapartida, a combinação de *P. viridis* e *B. caapi* resultou em menor atividade antimicrobiana, com CIM aumentada de 20 mg/mL, sugerindo que o uso combinado não foi vantajoso. Diante disso, os resultados destacam o grave impacto da resistência bacteriana na tríplice fronteira e a necessidade de alternativas terapêuticas. O extrato de *P. viridis* surge como uma opção promissora para o desenvolvimento de novos antimicrobianos, embora estudos adicionais sejam imprescindíveis para elucidar os compostos

ativos, mecanismos de ação e possíveis combinações com medicamentos convencionais. Em conjunto, as pesquisas enfatizam a relevância de monitoramento contínuo e de estratégias inovadoras no combate às cepas multirresistentes, especialmente em regiões de alta vulnerabilidade epidemiológica. Vale ressaltar que essa temática cumpre a tarefa 3.d dos Objetivos de Desenvolvimento Sustentável (ODS), proposta pela Organização das Ações Unidas (ONU), pois contribui para o desenvolvimento de estratégias de prevenção e gestão em saúde, com o intuito de assegurar bem-estar e proteção à saúde em um contexto global.

Palavras-chave: *Ayahuasca*. Fronteira trinacional. *Klebsiella pneumoniae*. *Banisteropsis caapi*. *Psychotria viridis. Terminalia catappa*.

BATISTA, Aline Cristiane Cechinel. **Bacterial resistance and therapeutic potential**: Carbapenemases and the use of medicinal plant extracts in the tri-border area (Brazil, Paraguay, and Argentina). Advisor: Daniela Dib Gonçalves. 2025. 125 p. Thesis (Doctorate degree in Animal Science with Emphasis on Bioactive Products) - Universidade Paranaense, Umuarama, 2025.

ABSTRACT

Bacterial resistance represents one of the greatest threats to global public health, especially when exacerbated by the spread of carbapenem-resistant strains, such as carbapenemase-producing *Klebsiella pneumoniae* (KPC and NDM). In this context, the triple border between Brazil, Paraguay, and Argentina, characterized by intense population mobility, is identified as a high-risk region for the development of epidemic outbreaks related to these multidrug-resistant strains. Thus, this study aims to investigate the resistance profile of carbapenemase producing *Klebsiella pneumoniae* (KPC and NDM) in an emergency hospital on the triple border between Brazil, Paraguay and Argentina, to understand the sociodemographic aspects of affected patients and to evaluate the efficacy of *Terminalia catappa, Banisteropsis caapi* and *Psychotria viridis* extracts against these resistant strains. A total of 48 cases of *K. pneumoniae* KPC and/or NDM were analyzed, where samples were mainly collected from intensive care units and showed significant resistance to various antibiotics such as penicillin (100%), cephalosporins (100%), carbapenems (100%), aminoglycosides (95.8%), and sulfonamides (95.8%), except for colistin (22.9%) and ceftazidime/tazobactam (39.6%). KPC strains (60.4%) were more prevalent, followed by NDM strains (27.1%) and co-resistance (12.5%). The clinical profile of the patients revealed most males, with an average age of 64 years, residing in Foz do Iguaçu and using the public health system. Regarding therapeutic potential, extracts of *Terminalia catappa* (7 copas), *Banisteropsis caapi*, and *Psychotria viridis* (*Ayahuasca*) were tested for antimicrobial activity against *K. pneumoniae* strains. *P. viridis* demonstrated the best efficacy, with a minimum inhibitory concentration (MIC) between 2.5 mg/mL and 5 mg/mL, while *B. caapi* showed very similar results. *T. catappa* had an MIC of 10 mg/mL. In contrast, the combination of *P. viridis* and *B. caapi* resulted in lower antimicrobial activity, with an increased MIC of 20 mg/mL, suggesting that the combined use was not advantageous. Therefore, the results highlight the severe impact of bacterial resistance in the triple border and the need for therapeutic alternatives. The extract of *P. viridis* emerges as a promising option for the development of new antimicrobials, although further studies are essential to elucidate the active compounds, mechanisms of action, and possible combinations with conventional medications. Together, the research emphasizes the relevance of continuous monitoring and innovative strategies in combating multidrug-resistant strains, especially in regions of high

epidemiological vulnerability. It is worth noting that this topic fulfills task 3.d of the Sustainable Development Goals (SDGs) proposed by the United Nations (UN), as it contributes to the development of prevention and health management strategies, aiming to ensure well-being and health protection in a global context.

Keywords: *Ayahuasca*. Bacterial resistance. Tri-nacional border. *Klebsiella pneumoniae*. Public health. 7 Copas.

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LISTA DE SIGLAS

ANVISA	Agência Nacional de Vigilância Sanitária
BGN	Bacilo Gram-negativo
BHI	Caldo *Brain Heart Infusion*
*Bla*KPC	Betalactamase KPC
*Bla*NDM	Betalactamase NDM
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEP	Comitê de Ética em Pesquisa
CIM	Concentração Inibitória Mínima
CLSI	*Clinical and Laboratory Standards Institute*
DMT	Dimetiltriptamina
FDA	*Food and Drug Administration*
KPC	*Klebsiella pneumoniae* Carbapenemase
MBL	Metalo-betalactamase
MDR	Multidroga resistente
MRSA	*Staphylococcus aureus* resistente à meticilina
NDM	Nova Delhi Metalo-betalactamase
ONU	Organização das Nações Unidas
OMS	Organização Mundial da Saúde
OXA-48	OXA-*type* Betalactamase
PDR	*Pan drug resistance*
PNPMF	Programa Nacional de Plantas Medicinais e Fitoterápicos
PNPIC	Política Nacional de práticas integrativas e complementares
PROSUP	Programa de suporte à Pós-graduação de Instituições de Ensino Particulares
rDNA	Ácido desoxirribonucleico recombinante
UNIPAR	Universidade Paranaense
UNILA	Universidade Federal da Integração Latino-Americana
VIM	Verona *Integron-encoded* metalo-betalactamase
XDR	Extensivamente resistente a medicamentos

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CAPÍTULO 1

REVISÃO DA LITERATURA

PERFIL DE RESISTÊNCIA ANTIMICROBIANA DE *Klebsiella pneumoniae* KPC E NDM EM REGIÃO DE TRÍPLICE FRONTEIRA E O POTENCIAL TERAPÊUTICO DE PLANTAS MEDICINAIS COMO ALTERNATIVA BIOATIVA.

O capítulo 1 foi editado de acordo com as normas da Associação Brasileira de Normas Técnicas - ABNT.

PERFIL DE RESISTÊNCIA ANTIMICROBIANA DE *Klebsiella pneumoniae* KPC E NDM EM REGIÃO DE TRÍPLICE FRONTEIRA E O POTENCIAL TERAPÊUTICO DE PLANTAS MEDICINAIS COMO ALTERNATIVA BIOATIVA.

1.1 Introdução

O surgimento e disseminação de microrganismos multidroga resistentes (MDR) têm se tornado uma séria ameaça à saúde pública global e ao que tudo indica, sem previsão de ser solucionada, podendo afetar indivíduos em qualquer momento da vida. Embora o desenvolvimento de resistência antimicrobiana seja um fenômeno natural dos microrganismos, esses mecanismos estão sendo potencializados por fatores externos como o mau uso ou prescrição inadequada dos antimicrobianos, por exemplo, facilitando assim a seleção, exposição e disseminação de cepas resistentes (LACEN/SC, 2016; BRASIL, 2020).

O saldo de óbitos atualmente registrado em decorrência de infecções por linhagens resistentes é de 700 mil indivíduos por ano (OPAS, 2020; OMS, 2022). Dados recentemente publicados pelo Grupo *Ad Hoc* de Coordenação Interagencial da Organização das Nações Unidas (ONU) sobre resistência antimicrobiana (IAGC) revelaram que, se providências não forem tomadas em relação a esse quadro, a resistência antimicrobiana poderá causar 10 milhões de mortes por ano até 2050 (IACG, 2020; TANG, MILLAR, MOORE, 2023).

Dentre os principais microrganismos causadores de doenças e associados a multirresistência, sabe-se que as enterobactérias, em especial a *Klebsiella pneumoniae* tem se destacado significativamente, principalmente no âmbito hospitalar, devido à sua elevada incidência e capacidade de adquirir e transferir informações genéticas que configuram mecanismos de multirresistência. Entre esses mecanismos, destaca-se a produção de carbapenemases, como a KPC (*Klebsiella pneumoniae* carbapenemase) e a NDM (Nova Delhi metalobetalactamase), enzimas que conferem resistência a antibióticos carbapenêmicos, frequentemente utilizados como última linha de tratamento. A presença dessas enzimas tem se tornado cada vez mais comum em infecções hospitalares, dificultando o manejo clínico e elevando taxas de morbidade e mortalidade (BATISTA, 2020). Por essa razão, *K. pneumoniae* foi enquadrada pela Organização Mundial da Saúde (OMS) como um patógeno de alta prioridade na busca por novos antimicrobianos (SANTOS, SECOLI, 2018; OPAS, 2025).

Diante desse cenário preocupante e da limitação terapêutica frente às bactérias resistentes, cresce o interesse por estratégias alternativas, como o uso de produtos naturais e

compostos bioativos extraídos de plantas medicinais. Nos países em desenvolvimento como o Brasil, tratamentos medicinais alternativos como a utilização de extratos de ervas consideradas medicinais são amplamente utilizados para o tratamento de diversas patologias. Na América Latina, a OMS reportou que 71% dos Chilenos e 40% dos Colombianos fazem uso de terapias alternativas utilizando plantas medicinais, além disso, muitos países Asiáticos têm utilizado esse tipo de tratamento mesmo tendo livre acesso às terapias ocidentais (BUSSMANN *et al.*, 2010).

Dessa forma, as plantas medicinais representam uma fonte notável e uma esperança para o tratamento de várias doenças, principalmente àquelas causadas por microrganismos patogênicos de difícil tratamento como os MDR. A diversidade química e biológica e potencial para produção de uma variedade de compostos biológicos promissores tem elevado as pesquisas por esses fitoterápicos, que além disso apresentam muitas vantagens dentre as quais destaca-se o baixo custo, fácil acessibilidade e reduzido efeito colateral (RIBEIRO *et al.*, 2018).

O Brasil é um país rico em biodiversidade, com mais de 45 mil espécies de plantas, o que compreende 20 a 22% do total de espécies no mundo. No entanto tal potencial farmacológico brasileiro é pouco explorado, levando em consideração o reduzido número de compostos naturais isolados da biodiversidade brasileira (MCTI, 2022).

Terminalia catappa é uma espécie de árvore de médio a grande porte, podendo chegar até a altura de 35 metros, popularmente conhecida como “amendoeira-da-praia”, “castanhola” ou “chapéu-de-sol”, devido à sua forma característica que proporciona sombra abundante. Pode ser encontrada mais facilmente em regiões tropicais, principalmente em áreas litorâneas do nordeste, sul e sudeste do Brasil, África e Ásia. É tradicionalmente utilizada como agente antinflamatório, antimicrobiano, hepatoprotetor, vermífugo, antioxidante, anticancerígeno e antidiabético (MWANGI, 2024).

O cipó *Banisteriopsis caapi* (mariri) e as folhas de *Psychotria viridis* (chacrona) juntas dão origem a conhecida bebida *Ayahuasca*, popularmente conhecida por suas propriedades alucinógenas e psicoativas e consumida por povos indígenas latino-americanos em rituais religiosos. No entanto, estudos recentes têm demonstrado efeitos positivos dessas plantas no tratamento de Alzheimer e na inibição antibacteriana, principalmente em cepas de microrganismos Gram negativos (ALVES, 2022).

Levando em consideração o crescente avanço da resistência bacteriana frente à produção de novos fármacos pela indústria farmacêutica, faz-se necessário o investimento em pesquisa

de novas fontes antimicrobianas eficazes como opção terapêutica para o tratamento de patologias causadas por microrganismos resistentes, especialmente em ambiente nosocomial.

1.2 Revisão de Literatura

1.2.1 *Enterobacterales* e as carbapenemases

As enterobactérias são bacilos Gram-negativos (BGN) pertencentes a família *Enterobacteriaceae* ordem das *Enterobacterales*. Embora possam ser encontrados distribuídos na natureza, atualmente, estão relacionados à casos de infecções nosocomiais. Dentre as principais bactérias dessa família, a *Klebsiella pneumoniae* tem se destacado por apresentar-se versátil em adquirir e transmitir mecanismos de resistência, facilitando sua disseminação (BATISTA, 2020).

A antibioticoterapia indiscriminada tem se tornado um grande desafio à saúde pública em todo o mundo, pois tem levado à proliferação de bactérias produtoras de enzimas de resistência, como as carbapenemases do tipo metalo-betalactamases (MBL) e as KPCs. Essas enzimas podem tornar a maior parte, ou todo o grupo dos betalactâmicos, inadequado para tratamento e, em associação com outros mecanismos, podem levar ao surgimento de cepas multirresistentes (MDR), extensivamente resistentes (XDR) e resistentes a todas as drogas (PDR) (CHELARU *et al.*, 2024).

De acordo com Batista (2020), essas carbapenemases realizam a quebra do anel betalactâmico dos antimicrobianos carbapenêmicos, inativando sua capacidade de impedir a síntese da parede celular bacteriana, característica essencial para sua ação antimicrobiana. Segundo a classificação de Ambler, elas estão distribuídas em três principais classes de acordo com o seu mecanismo molecular.

As metalo-betalactamases, como NDM (Nova Delhi metalo-betalactamase), VIM (Verona *Integron-encoded* metalo-betalactamase) e IMP (Imipenase metalo-betalactamase), utilizam íons de zinco em seus sítios ativos para catalisar a hidrólise do anel betalactâmico dos antimicrobianos, conferindo resistência a quase todos os betalactâmicos, exceto os monobactâmicos. O zinco facilita a ativação de uma molécula de água, que hidrolisa o anel betalactâmico através da quebra da ligação amida, inativando o antibiótico (ORTEGA-BALLEZZA *et al.*, 2024).

Por outro lado, as serina betalactamases, como a KPC e OXA-48, utilizam um resíduo de serina no sítio ativo. A serina nucleofílica ataca o anel betalactâmico, que é posteriormente

hidrolisado, liberando o antibiótico já inativado (ORTEGA-BALLEZZA *et al.*, 2024). A KPC pertence à classe A, que depende da presença de serina para atuar, enquanto a OXA-48 pertence à classe D, também conhecida como oxacilinases, que hidrolisam oxacilinas e carbapenêmicos, com uma menor eficiência comparada às metalo-betalactamases, mas são igualmente preocupantes em termos de resistência (ORTEGA-BALLEZZA *et al.*, 2024).

As infecções causadas por bactérias produtoras de carbapenemases, como *Klebsiella pneumoniae* produtora de KPC, geralmente estão associadas a elevadas taxas de mortalidade, que podem variar de 40% a 60%, dependendo do patógeno envolvido, do estado de saúde do paciente e da adequação do tratamento (MARTINEZ-OLIVA *et al.*, 2019). Sendo assim, o principal desafio clínico está na limitação das opções terapêuticas, já que muitas bactérias resistentes aos carbapenêmicos também são resistentes a outros antimicrobianos de última linha, como as cefalosporinas de terceira geração. Dessa forma, o tratamento dessas infecções frequentemente requer o uso de antimicrobianos como polimixinas, tigeciclina e fosfomicina, que apresentam eficácia limitada e estão associados a toxicidades significativas, como nefro e hepatotoxicidade. Ademais, a seleção inadequada de antimicrobianos pode agravar ainda mais o quadro clínico, aumentando as taxas de mortalidade, prolongando o tempo de internamento hospitalar e consequentemente onerando o serviço de saúde (SHEU *et al.*, 2019).

1.2.2 Resistência bacteriana em região de fronteira

A resistência bacteriana é um problema urgente de saúde pública e socioeconômico, de acordo com os últimos dados emitidos pela Organização Mundial de Saúde (2022) com 4,95 milhões de mortes associadas à resistência antimicrobiana em 2019, destas 1,27 milhões foram diretamente atribuíveis a ela. Além disso, a resistência é mais prevalente em países de baixa e média renda, apesar do menor consumo de antimicrobianos *per capita*. Algumas estimativas indicam que, até 2050, a resistência antimicrobiana poderá causar até 10 milhões de mortes por ano em todo o mundo (OMS, 2022).

A movimentação constante entre fronteiras, seja por turismo ou pelo transporte de culturas e animais, facilita a propagação global de microrganismos resistentes (MANAIA *et al.*, 2020). Em 2019, o tráfego aéreo global atingiu 4,5 bilhões de passageiros, enquanto 29,7 milhões de pessoas embarcaram em cruzeiros que frequentemente pararam em diferentes países. Além disso, cepas e genes resistentes adquiridos durante essas viagens podem permanecer nos indivíduos por até 12 meses, contribuindo ainda mais para o potencial de disseminação dessas resistências (COQUE, 2023).

Na região da Tríplice Fronteira, a circulação de pessoas nos postos aduaneiros reflete a intensa dinâmica migratória e comercial da região. Em 2024, somente no mês de janeiro, a Aduana da Ponte Internacional Tancredo Neves, que conecta o Brasil à Argentina, registrou um fluxo de 120.201 pessoas. Já na Aduana da Ponte Internacional da Amizade, principal ligação terrestre entre o Brasil e o Paraguai, o movimento foi ainda mais expressivo, com 169.619 registros de entrada e saída, consolidando-a como a fronteira mais movimentada do país. Considerando esses dados, Foz do Iguaçu registrou um total de 289.820 pessoas transitando pelas suas fronteiras internacionais em 2024, colocando o município como a terceira fronteira mais movimentada do Brasil, ficando atrás apenas das cidades de São Paulo e Rio de Janeiro. Esses números evidenciam a importância estratégica de Foz do Iguaçu como um dos principais corredores de fluxo migratório e comercial da América do Sul (POLÍCIA FEDERAL, 2025).

A situação crítica global de cepas resistentes a carbapenêmicos no Brasil, especialmente na região sul, incluindo o Paraná e as áreas de fronteira com o Paraguai e a Argentina, representa uma preocupação crescente para a saúde pública. O Brasil tem enfrentado surtos envolvendo KPC (*Klebsiella pneumoniae* carbapenemase) e NDM (Nova Delhi metalo-betalactamase), o que reflete um padrão observado em toda a América Latina (CHAGAS, RANGEL, DE SIMONE, 2024; KIFFER, *et al.*, 2023).

No Estado do Paraná – Brasil, hospitais têm relatado casos de infecções causadas por bactérias resistentes a carbapenêmicos, que representam um grande desafio para os sistemas de saúde. Ademais, a presença dessas cepas na fronteira entre Brasil, Paraguai e Argentina aumenta o risco de disseminação devido ao alto trânsito de pessoas e mercadorias na região, agravando a transmissão dessas cepas resistentes em contextos hospitalares e comunitários regionais (KIFFER, *et al.*, 2023).

A rápida disseminação de cepas resistentes ao longo dessas áreas fronteiriças requer uma resposta em conjunto entre os países, uma vez que o fenômeno da resistência antimicrobiana se propaga através de pacientes que cruzam as fronteiras em busca de tratamentos médicos ou turistas colonizados com essas cepas resistentes.

Um exemplo marcante foi descrito por Hernandez-Garcia *et al.* (2024) na Espanha, onde pacientes ucranianos transferidos após receberem assistência médica em seu país de origem apresentaram isolados de *Pseudomonas aeruginosa* ST773 produtores de NDM-1. Esses isolados compartilhavam mais de 99% de identidade genética e continham um repertório idêntico de genes de resistência, sugerindo uma disseminação clonal anterior ao atendimento em território espanhol.

Além disso, nesses mesmos pacientes, foi identificada a co-colonização por *Klebsiella pneumoniae* ST147, produtora simultânea de NDM-1 e OXA-48, demonstrando a complexidade epidemiológica associada à disseminação de genes de resistência. O clone ST147 destaca-se por sua capacidade de abrigar múltiplas carbapenemases, como KPC, NDM e OXA-48, devido à presença de elementos genéticos móveis que facilitam a aquisição e transmissão desses genes. Evidências epidemiológicas indicam ainda a circulação de plasmídeos distintos portadores dos genes *bla*NDM-1 e *bla*OXA-48 em *K. pneumoniae*, resultando em padrões variados de disseminação e reforçando a necessidade premente de vigilância microbiológica contínua e coordenada (HERNANDEZ-GARCIA *et al.*, 2024).

No contexto da fronteira EUA-México, Benoit *et al.* (2014) documentaram tendências de resistência antimicrobiana em isolados clínicos de oito hospitais localizados em seis cidades ao longo dessa região entre os anos de 2000 e 2006. A resistência mais elevada foi observada em *Staphylococcus aureus* resistente à oxacilina (MRSA), com uma prevalência de 45,7%, seguida por *P. aeruginosa* resistente às quinolonas (22,3%) e *Escherichia coli* resistente às quinolonas (15,6%). Os autores sugerem que esses níveis de resistência estão fortemente associados ao uso abusivo e descontrolado de antimicrobianos, uma vez que nessa região é comum que pacientes cruzem a fronteira em busca de atendimento médico e adquiram antimicrobianos sem prescrição adequada. Essa prática, aliada ao fácil acesso a medicamentos no lado mexicano e ao uso inadequado, favorece a seleção e propagação de cepas multirresistentes, demonstrando a necessidade de ações coordenadas entre os países para conter a disseminação desses patógenos.

1.2.3 Plantas medicinais

As plantas constituem uma fonte bastante antiga de cura e tratamento para as mais diversas patologias que têm assolado a humanidade, e até mesmo nos dias atuais, permanecem invictas contribuindo para o desenvolvimento de novos medicamentos. O que pode ser evidenciado através das ações do *Food and Drug administration* (FDA) que aprovou 16 novos medicamentos derivados de plantas nos anos de 2001 a 2010 (BOLZANI *et al.*, 2012).

O uso terapêutico das plantas medicinais na saúde humana é historicamente construído pela sabedoria do senso comum por meio da articulação da cultura e saúde, sendo utilizada desde milhares de anos antes de Cristo (BRUNING, MOSEGUI, VIANNA, 2012).

A mais antiga evidência escrita do uso de plantas medicinais foi encontrada em uma laje de argila suméria de Nagpur, com aproximadamente 5.000 anos, que cita em torno de 250

plantas. Algumas destas plantas são utilizadas até os dias atuais, como a papoula (*Papaver somniferum*) e a maconha (*Cannabis sativa*), as quais o uso tem sido descrito por pelo menos 4.000 anos (AZEVEDO, 2018).

A utilização de plantas medicinais é o resultado do acúmulo secular de conhecimentos empíricos sobre a ação delas por diversos grupos étnicos resultando na origem de uma medicina tradicional. No Brasil, além dos conhecimentos tradicionais indígenas, as contribuições trazidas nesse campo do conhecimento pelos imigrantes e pelos escravos tiveram importância significativa no surgimento de uma medicina popular rica baseada na utilização da biodiversidade vegetal (MACHADO, VARGAS, 2018).

O Brasil tem uma das maiores biodiversidades do planeta, e muitas classes de princípios ativos têm sido isoladas de plantas medicinais brasileiras. Isto se dá pelo vasto território nacional, que abrange diferentes biomas, e possui mais de 56.000 espécies de plantas. Estima-se que existam aproximadamente 500 mil espécies de plantas no mundo, sendo que cerca de 30% apresentam potencial terapêutico. Esse cenário evidencia a biodiversidade brasileira como um campo vasto e promissor para a pesquisa científica (AZEVEDO, 2018).

O uso de plantas medicinais é uma prática comum entre a população brasileira e reflete uma herança cultural passada entre as gerações e fruto da miscigenação do povo. Dados da OMS, citada por Vieira *et al.* (2016), revelam que 80% da população dos países em desenvolvimento utiliza-se da medicina tradicional para manutenção de suas necessidades básicas de saúde.

No entanto, de acordo com Azevedo *et al.* (2018), com o surgimento da indústria farmacêutica houve um declínio no uso terapêutico de plantas medicinais, observado no final do século XIX e começo do século XX. Entretanto, recentemente tem despertado um interesse em relação ao uso de plantas medicinais, principalmente devido aos expressivos efeitos colaterais causados pelas drogas sintéticas e ao aumento das contraindicações delas. Diante disso, pesquisadores brasileiros e companhias farmacêuticas brasileiras têm voltado seus esforços para o estudo de plantas medicinais nativas e seus princípios ativos.

No Brasil, políticas como o Programa nacional de plantas medicinais e fitoterápicos (PNPMF) e a Política nacional de práticas integrativas e complementares (PNPIC) integram fitoterápicos ao Sistema Único de Saúde (SUS), promovendo seu uso seguro e regulamentado pela Agência Nacional de Vigilância Sanitária (ANVISA). Essas iniciativas valorizam a biodiversidade, o conhecimento tradicional e contribuem para a preservação cultural, o

desenvolvimento econômico sustentável e a proteção ambiental (CACCIA-BAVA *et al.*, 2017; CARVALHO *et al.*, 2011; PALHARES *et al.*, 2021).

1.2.4 Metabólitos secundários

Os metabólitos produzidos pelos vegetais podem ser divididos em dois grupos: um conjunto de reações conhecidas como metabolismo primário e outro caracterizado por reações de metabolismo secundário. Este último possui funcionalidades, mas que não estão diretamente ligadas ao crescimento e desenvolvimento do organismo (COOPER e NICOLA, 2015).

Em teoria, todas as plantas são capazes de produzir tais metabólitos, que ao longo do seu ciclo evolutivo, acabaram desenvolvendo mecanismos adaptativos de sobrevivência, ou seja, os metabólitos secundários presentes nas plantas medicinais desempenham papéis fundamentais na defesa contra estresses ambientais e na interação com organismos externos (VIZZOTTO, KROLOW e WEBER, 2010).

Esses metabólitos podem ser divididos em três grupos quimicamente distintos: os terpenos, os fenólicos e os compostos nitrogenados. Os alcaloides se encontram no grupo dos compostos nitrogenados, e são constituídos por uma família com mais de 15.000 variedades estruturais desses produtos secundários, dentre os quais aproximadamente 20% são encontrados em espécies de plantas vasculares (AZEVEDO, 2018).

Os terpenos são a maior classe de metabólitos secundários, formados a partir de isopreno, fazem parte deste grupo os monoterpenos, diterpenos, sesquiterpenos e triterpenos compondo os óleos essenciais. Na planta, conferem proteção contra herbívoros e patógenos, além de atrair polinizadores e dispersores de sementes por seus aromas. Apresentam propriedades antimicrobianas, antinflamatórias, ansiolíticas e analgésicas (CÂMARA *et al.*, 2024).

1.2.5 *Terminalia catappa*

Popularmente conhecida como amendoeira-da-praia, “castanhola” ou “chapéu-de-sol”, caracterizada por uma copa ampla ereta, casca cinza e lisa, e ramos dispostos horizontalmente, suas folhas são grandes, verdes escuras, brilhantes, em formato oval e elípticas que mudam de coloração para tons de marrom, dourado, vermelho ou amarelo antes de cair. Produtora de frutos alongados com uma única semente em seu interior, *Terminalia catappa* é uma árvore tropical, pertencente à família *Combretaceae,* é uma planta nativa do Sudeste Asiático, incluindo países

como Malásia, Índia e Filipinas e geralmente encontrada em regiões costeiras como o litoral do nordeste, sul e sudeste do Brasil, África e Ásia (HABIBBULAH *et al.*, 2023; MWANGI, 2024).

Podendo chegar até 35 metros de altura, toda a planta (folhas, casca e sementes) é rica em compostos bioativos como taninos, flavonoides e alcaloides. E tais metabólitos conferem a esse material vegetal propriedades antioxidantes, antinflamatórias, antimicrobianas, antidiabéticas e hepatoprotetoras, motivo pelo qual apresenta ampla utilização na medicina tradicional em diferentes culturas (ZANATTA *et al.*, 2023).

Figura 1: Árvore de 7 copas (*Terminalia catappa*)

Fonte: SETE COPAS (2025).

T. catappa de um modo geral apresenta potencial etnofarmacológico, visto que todas as suas partes podem ser utilizadas medicinalmente. Desde a casca (desinteria), frutos (antimicrobianos) e principalmente suas folhas que podem ser utilizadas para diversas finalidades, tanto na prevenção de doenças quanto no tratamento delas, através de propriedades antioxidantes, antinflamatórias, antibacterianas, cardioprotetoras, hepatoprotetoras, anticancerígenas e até mesmo quimiopreventivas (HABIBBULAH *et al.*, 2023).

Do ponto de vista antibacteriano, os compostos bioativos de *T. catappa* atuam de maneira sinérgica para inibir o crescimento bacteriano. Os flavonoides, por exemplo, interferem na formação de biofilmes bacterianos, enquanto os taninos precipitam proteínas microbianas, inibindo a adesão bacteriana. Os terpenoides agem desestabilizando a membrana celular das bactérias, e as saponinas têm a capacidade de romper essas membranas, levando à lise celular.

Em conjunto, esses compostos tornam *T. catappa* uma promissora fonte natural de substâncias com potencial antibacteriano. (MWANGI *et al.*, 2024).

Estudos demonstram que o extrato metanólico de *T. catappa* mostrou-se eficaz contra cepas resistentes como *Escherichia coli*, *Staphylococcus aureus* e *Klebsiella pneumoniae*, embora tais estudos não contemplem cepas relacionadas à resistência aos carbapenêmicos, eles indicam que a planta é uma promissora fonte de compostos antimicrobianos para o combate a infecções por enterobactérias e cepas multirresistentes (SOWMYA, 2021).

1.2.6 *Psychotria viridis e Banisteropsis caapi*

Psychotria viridis é uma planta arbustiva de pequeno porte (possui entre 2 e 3 metros de altura) pertencente à família *Rubiaceae*. Também denominada "Rainha" ou "Chacrona", ela está presente principalmente na Floresta Amazônica, bem como no México e nas Antilhas, Bolívia, Argentina e sudeste do Brasil, no entanto, por estar relacionada a questões religiosas, podem ser encontradas plantações em várias partes do mundo. A espécie possui em suas folhas o alcaloide dimetiltriptamina (DMT), uma amina indólica que é considerada um potente alucinógeno (AZEVEDO, 2018).

Figura 2*: Psychotria viridis*

Fonte: DOMÍNGUEZ-CLAVÉ *et al.* (2016).

Banisteriopsis caapi, também conhecida como "Jagube" ou "Mariri", é um tipo de cipó ou trepadeira gigante pertencente à família *Malpighiaceae*. Presente nas regiões Norte (Acre, Amazonas, Pará e Rondônia) e Centro-Oeste (Mato Grosso), em um tipo de vegetação de floresta de terra firme, tendo seu domínio fitogeográfico na região Amazônica. Neste cipó são

encontrados alcaloides beta-carbonílicos, sendo os principais: harmina, harmalina e tetrahidroharmina, capazes de inibir a enzima Monoamina oxidase tipo A (MAO-A), enzima responsável pela degradação da serotonina endógena (PENA, 2013).

Figura 3: *Banisteropsis caapi*

Fonte: DOS (2025).

A junção das folhas da *P viridis* com os caules da *B. caapi* dão origem a uma bebida chamada *Ayahuasca* e promovem uma combinação sinérgica, pois o cipó possui alcaloides beta-carbonílicos que realizam a metabolização da DMT, impedindo que esse alcaloide seja ativado quando administrado por via oral (AZEVEDO, 2018; ESTRELLA-PARRA, ALMANZA-PEREZ, ALARCON-AGUILAR, 2019).

Esta bebida tem sido amplamente utilizada por tribos indígenas em rituais religiosos como fonte de obter crescimento pessoal e conexão espiritual, mas também em cenários recreativos, tornando-se uma grande preocupação para a saúde pública devido à sua toxicidade. No entanto, muitos estudos ainda estão sendo realizados para a sua utilização no tratamento médico, principalmente no campo das doenças psiquiátricas (SIMÃO *et al.*, 2020).

1.2.7 Considerações Finais

Diante do agravamento da resistência antimicrobiana e a dificuldade no tratamento de infecções causadas por microrganismos resistentes, torna-se indispensável a busca por novas opções terapêuticas. As plantas medicinais, como *Terminalia catappa, Banisteriopsis caapi e Psychotria viridis*, têm demonstrado potencial antimicrobiano promissor, podendo representar uma fonte para novos compostos bioativos.

Nesse contexto, destaca-se a urgência em encontrar alternativas eficazes contra bactérias produtoras de carbapenemases, especialmente KPC e NDM, que estão entre os principais mecanismos de resistência a antibióticos de última linha e têm sido responsáveis por surtos hospitalares de difícil controle.

Considerando a biodiversidade brasileira e a necessidade de soluções inovadoras, a exploração científica desses recursos naturais pode contribuir expressivamente para o desenvolvimento de estratégias terapêuticas mais acessíveis e eficazes, sobretudo em contextos hospitalares e regiões fronteiriças, onde a disseminação de cepas resistentes representa um grande desafio à saúde pública.

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1.4 Objetivo

Investigar o perfil de resistência de *Klebsiella pneumoniae* produtora de carbapenemases (KPC e NDM) em um hospital de urgência na tríplice fronteira entre Brasil, Paraguai e Argentina, compreender os aspectos sociodemográficos dos pacientes afetados e avaliar a eficácia dos extratos de *Terminalia catappa, Banisteriopsis caapi e Psychotria viridis* contra essas cepas resistentes.

CAPÍTULO 2

ARTIGOS

CARBAPENEMASES: RESISTANCE PROFILE IN NA INTERNATIONAL BORDER HOSPITAL (Brazil X Paraguay X Argentina)

Artigo editado de acordo com as normas de publicação da Revista Ciência & Saúde Coletiva – ISSN 1678-4561.

CARBAPENEMASES: RESISTANCE PROFILE IN NA INTERNATIONAL BORDER HOSPITAL (Brazil X Paraguay X Argentina)

CARBAPENEMASES: PERFIL DE RESISTÊNCIA EM UM HOSPITAL DE FRONTEIRA INTERNACIONAL (Brasil x Paraguai x Argentina)

CARBAPENEMASAS: PERFIL DE RESISTENCIA EN UN HOSPITAL DE FRONTERA INTERNACIONAL (Brasil x Paraguay x Argentina)

ABSTRACT

The aim of this study was to investigate bacterial resistance in infections caused by *Klebsiella pneumoniae* producing carbapenemases (KPC and NDM) in an emergency hospital located in the tri-border region of Brazil–Paraguay–Argentina. A total of 48 samples positive for KPC and/or NDM were analyzed, identified through microbiological and molecular methods. Clinical data were obtained from hospital reports and organized in spreadsheets. It was observed that 58.3% of the patients were male, with a mean age of 64 years, all residing in Foz do Iguaçu; 62.5% used the public health system. Most samples originated from intensive care units (47.9%). The isolates showed 100% resistance to penicillins, cephalosporins, and carbapenems, as well as high resistance to aminoglycosides and sulfonamides (95.8%). The lowest resistance

rates were to colistin (22.9%) and ceftazidime/tazobactam (39.6%). KPC-producing strains were the most prevalent (60.4%), followed by NDM (27.1%) and co-producing strains (12.5%). It is concluded that there was a predominance of elderly patients, users of the public health system, and ICU admissions, with a higher prevalence of KPC-mediated resistance. The study reinforces the importance of epidemiological surveillance and public policies aimed at containing the dissemination of resistant microorganisms in regions with intense population movement.

Keywords: Carbapenemases; Tri-border region; *Klebsiella pneumoniae*; KPC; NDM**.**

RESUMO

O objetivo deste estudo foi investigar a resistência bacteriana em infecções por *Klebsiella pneumoniae* produtoras de carbapenemases (KPC e NDM) em um hospital de urgência da tríplice fronteira Brasil–Paraguai–Argentina. Foram analisadas 48 amostras positivas para KPC e/ou NDM, identificadas por métodos microbiológicos e moleculares. Os dados clínicos foram obtidos de relatórios hospitalares e organizados em planilhas. Observou-se que 58,3% dos pacientes eram homens, com média de idade de 64 anos, todos residentes em Foz do Iguaçu; 62,5% utilizaram o SUS. As amostras eram majoritariamente de UTI (47,9%). Os isolados apresentaram resistência de 100% a penicilinas, cefalosporinas e carbapenêmicos, além de alta resistência a aminoglicosídeos e sulfonamidas (95,8%). A menor resistência foi à colistina (22,9%) e à ceftazidima/tazobactam (39,6%). Cepas KPC foram mais prevalentes (60,4%), seguidas por NDM (27,1%) e por co-produtoras (12,5%). Conclui-se que houve predominância de pacientes idosos, usuários do SUS e internados em UTI, com maior prevalência de resistência mediada por KPC. O estudo reforça a importância da vigilância epidemiológica e de

políticas públicas para conter a disseminação de microrganismos resistentes em regiões de intensa circulação populacional.

Palavras-chaves: Carbapenemases; Fronteira trinacional; *Klebsiella pneumoniae;* KPC; NDM.

RESUMEN

El objetivo de este estudio fue investigar la resistencia bacteriana en infecciones por *Klebsiella pneumoniae* productoras de carbapenemasas (KPC y NDM) en un hospital de urgencias ubicado en la triple frontera Brasil–Paraguay–Argentina. Se analizaron 48 muestras positivas para KPC y/o NDM, identificadas mediante métodos microbiológicos y moleculares. Los datos clínicos se obtuvieron de informes hospitalarios y se organizaron en hojas de cálculo. Se observó que el 58,3% de los pacientes eran hombres, con una edad media de 64 años, todos residentes en Foz do Iguaçu; el 62,5% utilizó el sistema público de salud. La mayoría de las muestras procedía de unidades de cuidados intensivos (47,9%). Los aislamientos mostraron resistencia del 100% a penicilinas, cefalosporinas y carbapenémicos, además de alta resistencia a aminoglucósidos y sulfonamidas (95,8%). La menor resistencia se observó frente a colistina (22,9%) y ceftazidima/tazobactam (39,6%). Las cepas productoras de KPC fueron las más prevalentes (60,4%), seguidas por NDM (27,1%) y las co-productoras (12,5%). Se concluye que hubo predominio de pacientes ancianos, usuarios del sistema público de salud e internados en UCI, con mayor prevalencia de resistencia mediada por KPC. El estudio refuerza la importancia de la vigilancia epidemiológica y de políticas públicas dirigidas a contener la diseminación de microorganismos resistentes en regiones de intensa circulación poblacional.

Palabras clave: Carbapenemasas; Frontera trinacional; *Klebsiella pneumoniae*; KPC; NDM.

INTRODUCTION

Bacterial resistance is an extremely serious concern that has reached alarming proportions, especially in hospital settings, and is a public health challenge on a global scale.[1-2]. According to the World Health Organization, projections indicate that by 2050, if no effective measures are taken to contain this threat, infections caused by pathogenic microorganisms could result in the deaths of up to 10 million people annually worldwide. Furthermore, this problem could cause economic damage of catastrophic magnitude and push up to 24 million people into extreme poverty[3-6].

Factors such as the indiscriminate use of antibiotics, inappropriate prescriptions and treatment failures have contributed to accelerating the process of bacterial resistance and this allows bacteria, including enterobacteria, which are capable of carrying mobile genetic elements, such as plasmids, to acquire and transfer resistance mechanisms to other enterobacteria easily and quickly[7-8].

Carbapenemases are enzymes produced by bacteria that can deactivate or hydrolyze antibiotics of the beta-lactam class, which includes carbapenems (such as imipenem, meropenem, and ertapenem)[5]. This has resulted in a significant increase in human mortality rates, as this form of resistance drastically limits therapeutic options for treating infections, with carbapenems being considered the last line of defense in treating infections, making resistance to them a critical threat to public health[9].

The mortality rate linked to infections caused by enterobacteria, such as *Klebsiella pneumoniae*, for example, can reach up to 75%, varying according to the age and health condition of the population under analysis and among the different types of carbapenemases documented, from an epidemiological point of view, class A carbapenemases (KPC) and class B carbapenemases (NDM) are extremely relevant due to their rapid dissemination capacity[3].

Around 70,800 vehicles and 150,000 people travel across the Triple Frontier daily. Foz do Iguaçu is located in the far west of the State of Paraná, Brazil, in a region that shares borders with two countries, Paraguay and Argentina. The high mobility of the population in this area creates an environment prone to the easy transport of pathogenic microorganisms from one place to another, increasing the risk of epidemic occurrences[10].

Epidemiological surveillance of carbapenemase-producing strains is essential due to the high mortality associated with them and their rapid dissemination via plasmids. In this context, this study aimed to investigate the resistance profile of *Klebsiella pneumoniae* (KPC and NDM) in a hospital in the triple border region (Brazil, Paraguay, and Argentina) and to analyze the sociodemographic aspects of patients, to support prevention, control and management strategies in public health.

METHODOLOGY

Ethical aspects

This project was approved by the Research Ethics Committee (CEP) of Universidade Paranaense – UNIPAR, under opinion number 5,171,267. All bacterial strains used in this research were obtained as part of the clinical routine of the hospital where the study was conducted. The samples analyzed came from routine examinations of hospitalized patients, recorded in hospital reports, and corresponded to positive cultures of *Klebsiella pneumoniae* producing KPC or NDM carbapenemases.

Study location

The study was conducted at a Private Hospital in the city of Foz do Iguaçu in the State of Paraná - Brazil. The institution has 202 inpatient beds, including 35 intensive care beds, with an average of 923 hospitalizations per month and approximately 398 surgeries per year.

Samples

It was composed of blood, urine, rectal swab, tracheal secretion and tissue samples from individuals who sought hospital medical care for some health disorder and presented positive microbial culture results for carbapenemase-producing *Enterobacterales*, *Klebsiella pneumoniae* carbapenemase (KPC) or New Delhi metallo-betalactamase (NDM) in the period from December 2021 to December 2022.

Research Instrument

The information was collected through a data report prepared by the Hospital's own IT department, to prevent access to the patient's medical records, protecting their information.

The research instrument included demographic data (age, sex, education level, and city of origin) and epidemiological data (resistance mechanism, hospitalization sector, clinical outcome, and bacterial resistance profile), organized in a Microsoft Excel 2010 spreadsheet.

Collection of data from laboratory analyses

Microbiology

Microbiological analyses were performed in the hospital laboratory, using the automated Vitek 2 system (Biomerieux®) for biochemical identification and determination of the minimum inhibitory concentration. *Klebsiella pneumoniae* isolates resistant to penicillins, cephalosporins, and carbapenems were selected. Identification occurred using GN cards, while

cards 238 and 239 were applied to antibiograms of urinary samples and other biological materials, respectively. Resistance was defined by MIC ≥ 2 μg/mL for meropenem/imipenem and ≥ 1 μg/mL for ertapenem, according to guidelines from ANVISA (Technical Note 01/2013) and the Brazilian Committee for Antimicrobial Susceptibility Testing. In addition, complementary tests were performed, including the Resist 3 OKN immunochromatographic test (CorisBioconcept®), to identify KPC, NDM, and OXA-48.

Molecular Biology

All isolates were identified by real-time PCR with primers specific for the blaKPC and blaNDM genes, using Taqman chemistry, with an endogenous bacterial control of the 16S rDNA region, which is highly conserved in the species[4].

The extraction of genetic material was performed using the thermal lysis technique, with bacterial colonies resuspended in 200 μL of ultrapure water in sterile microtubes, free of DNAse and RNAases, and incubated at 99°C for 10 minutes[4].

The investigated genes were amplified using the following primers and probes (Table 1), as described by Batista[4]:

Table 1 – List of primers and probes used to perform the molecular identification of *K. pneumoniae* isolates in biological samples collected from patients hospitalized at an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

Gene target	Primer/Probe	Sequence (5' → 3')
KPC	F Primer	GGC CGC CGT GCA ATA C
	R Primer	GCC GCC CAA CTC CTT CA

	Probe	FAM-TG ATA ACG CCG CCG CCA ATT TGT-BHQ
NDM	F Primer	GAC CGC CCA GAT CCT CAA
	R Primer	CGC GAC CGG CAG GTT
	Probe	HEX-TG GAT CAA GCA GGA GAT-BHQ
16S rRNA	F Primer	TGG AGC ATG TGG TTT AAT TCG A
	R Primer	TGC GGG ACT TAA CCC AAC A
	Probe	CY5-CA CGA GCT GAC GAC ARC CAT GCA-BHQ

Subtitles: F – Forward; R – Reverse

Real-time PCR was performed with the QuantStudio 7 Flex - Real time PCR System (Thermofisher®) and the Luna® Universal qPCR Master Mix kit (Biolabs®). Cycling conditions consisted of 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C[4].

RESULTS

Forty-eight positive cultures of *K. pneumoniae* producing KPC and/or NDM were identified. Among the patients, 58.3% were male and 41.7% were female, with an average age of 64 years. The majority lived in Foz do Iguaçu (87.5%), while 10.4% were from other municipalities in Paraná and 2.1% from Paraguay. Regarding education, 50% did not provide information; among the others, 20.8% had completed elementary education, 16.7% had completed high school, 6.25% had incomplete elementary education, and 6.25% had incomplete high school. Regarding health coverage, 62.5% were covered by the SUS, 33.3%

by health plans, and 4.2% by private services. Samples were collected mainly in ICUs (47.9%), followed by inpatient wards (45.8%) and emergency departments (6.25%).

Table 2 - Sociodemographic characteristics and care profile of patients included in the study at an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

Characteristic		**n**	**%**
Total samples		48	100%
Gender	Male	28	58.3%
	Female	20	41.7%
Average age	64 years (19-84)		
Residence	Foz do Iguaçu	42	87.5%
Other municipalities in Paraná		5	10.4%
Paraguay		1	2.1%
Education	Not informed	24	50%
	Complete elementary school	10	20.8%
	Completed high school	8	16.7%
	Incomplete elementary school	3	6.25%
	Incompleted high school	3	6.25%
Health coverage	SUS	30	62.5%

	Other plans	16	33.3%
	Particular	2	4.2%
Sample collection site	ICU	23	47.9%
	Inpatient ward	22	45.8%
	EC/ER	3	6.25%

Subtitle: SUS – Unified Health System; ICU – Intensive Care Unit; EC - Emergency Care; ER – Emergency Room.

Rectal swabs were the most common samples (56.3%), followed by urine samples (22.9%), tracheal secretions (10.4%), and blood cultures (6.3%). Additionally, there were also a smaller number of tissue (2.1%) and catheter tip (2.1%) samples that exhibited resistance (Table 3).

Table 3- Incidence of resistance mechanisms by *K. pneumoniae* KPC, NDM, and Co-resistance (KPC/NDM) in biological samples collected from patients hospitalized at an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

	Urine	**Tracheal Secretion**	**Tissue**	**Catheter Tip**	**Blood culture**	**Rectal swab**	**No.**	**%**
***K. pneumoniae* KPC**	6	3	1	1	3	15	29	**60.4**
***K. pneumoniae* NDM**	4	2	0	0	0	7	13	**27.1**

K. pneumoniae **KPC/NDM**	1	0	0	0	0	5	6	**12.5**
TOTAL	**11**	**5**	**1**	**1**	**3**	**27**	**48**	**100.0**

Subtitle: *K. pneumoniae – Klebsiella pneumoniae*; KPC – *Klebsiella pneumoniae* carbapenemase; NDM – New Delhi Metalo-betalactamase.

Regarding the resistance profile, the KPC enzyme was predominant in all biological samples (60.4%), compared to the NDM enzyme (27.1%). Furthermore, co-resistance (12.5%) was identified less frequently than the other types of resistance, as also demonstrated in Table 4.

Table 4 - Distribution of positive samples for *K. pneumoniae* KPC, NDM, and Co-resistance (KPC/NDM) in biological samples collected from patients hospitalized at an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

	KPC	**NDM**	**KPC/NDM**	**Nº**	**%**
Urine	6	4	1	11	**22.9**
Tracheal Secretion	3	2	0	5	**10.4**
Tissue	1	0	0	1	**2.1**
Catheter Tip	1	0	0	1	**2.1**
Blood culture	3	0	0	3	**6.3**
Rectal swab	15	7	5	27	**56.3**
TOTAL	29	13	6	48	**100.0**

Legenda: *K. pneumoniae – Klebsiella pneumoniae*; KPC – *Klebsiella pneumoniae* carbapenemase; NDM – New Delhi Metalo-betalactamase.

The antimicrobial resistance profile was widely elevated among the isolates analyzed, except for ceftazidime/tazobactam (39.6%) and colistin (22.9%), which presented lower resistance rates compared to the other drugs tested.

The tissue sample showed concomitant sensitivity to both antibiotics, while the isolates from the catheter tip and blood cultures revealed sensitivity restricted to ceftazidime/tazobactam.

In urinary samples, universal resistance to β-lactams, quinolones, and aminoglycosides was observed; however, sensitivity was observed in one sample to sulfonamide, in eight (72.7%) to colistin, and in six (54.5%) to ceftazidime/tazobactam.

Among the isolates from tracheal secretion, two (40%) showed sensitivity to amikacin, while three (60%) were sensitive to both ceftazidime/tazobactam and colistin, maintaining resistance to the other classes tested.

Finally, the isolates obtained from rectal swabs showed high levels of sensitivity to colistin (88.9%), followed by ceftazidime/tazobactam (55.6%) and sulfonamide (3.7%), although they were resistant to all other antimicrobials evaluated.

Table 5 - Resistance profile of *K. pneumoniae* strains in biological samples collected from patients hospitalized at an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

ANTIBIOTICS	Urine	Tracheal Secretion	Tissue	Catheter Tip	Blood culture	Rectal swab	KPC	NDM	KPC/NDM	No.	%
Amox/ Clavulanate	11	5	1	1	3	27	29	13	6	48	**100**
Amikacin	11	**3**	1	1	3	27	29	**11**	6	46	**95.8**
Cefepime	11	5	1	1	3	27	29	13	6	48	**100**
Ceftriaxone	11	5	1	1	3	27	29	13	6	48	**100**
Ciprofloxacin	11	5	1	1	3	27	29	13	6	48	**100**
Gentamicin	11	5	1	1	3	27	29	13	6	48	**100**
Ertapenem	11	5	1	1	3	27	29	13	6	48	**100**
Meropenem	11	5	1	1	3	27	29	13	6	48	**100**
Imipenem	11	5	1	1	3	27	29	13	6	48	**100**
Ceftazidime/ Tazo	**5**	**2**	**0**	**0**	**0**	**12**	**0**	13	6	19	**39.6**
Colistin	**3**	**2**	**0**	1	**2**	**3**	**4**	**7**	**0**	11	**22.9**

SUT	10	5	1	1	3	26	29	**11**	6	46	**95.8**

Subtitle: Amox/ Clavulanate – Amoxicillin/ Clavulanate; Ceftazidime/ Tazo – Ceftazidime/ Tazobactam; SUT – Sulfazotrim/ Trimethoprim.

According to the observed resistance profile, colistin stood out as the antibiotic with the highest sensitivity, followed by ceftazidime/tazobactam. On the other hand, beta-lactams showed a 100% resistance rate in these strains, while the aminoglycoside amikacin, combined with sulfonamide, demonstrated a 95.8% resistance rate (Table 5).

In the case of KPC samples, total resistance to all beta-lactams, aminoglycosides, quinolones, and sulfonamides was observed. Only four (13.8%) samples demonstrated resistance to colistin, and no strains showed resistance to ceftazidime/tazobactam.

On the other hand, strains carrying the NDM enzyme exhibited resistance to beta-lactams and ceftazidime/tazobactam. Only two (15.4%) strains showed sensitivity to quinolones and sulfonamides, while six of them showed sensitivity to colistin.

In contrast, strains with co-resistance revealed comprehensive resistance to all antibiotics except colistin.

DISCUSSION

Given the limited progress in developing new drugs in response to the rapid spread of resistant microorganisms, studies like this are essential for expanding knowledge and supporting containment strategies, including improvements in institutional management and hospital conditions.

In border areas, the movement of people and goods is intense, which can facilitate the spread of resistant microorganisms to different geographic regions. Therefore, a thorough understanding of antimicrobial resistance and the implementation of effective hospital infection control strategies are of paramount importance in these areas. Furthermore, international collaboration and coordination between the health systems of different countries in border regions are essential to prevent outbreaks of drug-resistant infections and protect public health as a whole[10].

Forty-eight microbial cultures positive for *K. pneumoniae* producing the carbapenemase enzymes KPC and/or NDM were identified during the period covered by the research.

Within this group, there was a male majority (58.3%) compared to females (41.7%). The high proportion of men infected by *K. pneumoniae* corroborated the findings of several studies, such as Mariappan, Sekar, and Kamalanathan[13], Chen et al.[14] and Stewart et al.[15], all of which presented the following results: 66.7%, 69.4%, and 60% successively. According to Dias, Brouwer, and van de Beek[16], this trend can be attributed to hormonal variations and distinct immunological responses between men and women, giving men a possible advantage in terms of survival. Furthermore, Adrie et al.[17] suggest that women are less likely than men to undergo intensive medical evaluations and invasive procedures related to heart disease. Even though they are more likely to recommend and adhere to long-term ongoing health care, they demonstrate resistance to acute care.

Thus, the predominance of males among cases (58.3%) may suggest that there is a gender predisposition to these infections or may reflect differences in exposure or health care between the sexes[18-19]. However, further research is needed to better understand this discrepancy.

The average age of patients was around 64 years, with the highest concentration of cases occurring in individuals residing in the city of Foz do Iguaçu (87.5%), while another five cases (10.4%) were citizens residing in different municipalities in the state of Paraná; only one (2.1%) case was a resident in Paraguay.

Other studies, such as Chen et al.[14] and Stewart et al.[15], reported mean values very similar to those found in this study, which suggests a direct relationship with the profile of patients admitted to each hospital unit. Furthermore, the average age of affected patients (64 years) highlights the importance of considering the age range when analyzing these infections,

since elderly patients often have more fragile immune systems and may be more susceptible to bacterial infections[20].

The fact that most cases occurred in Foz do Iguaçu residents (87.5%) may be attributed to the acute hospital nature of the study, which primarily attracts local patients in emergencies. Studies focusing on microbial resistance are still scarce in this region; however, data published by the Health Surveillance Agency[21] indicate Foz do Iguaçu as the sixth municipality in Paraná in the number of notifications of healthcare-associated infections (HAIs). The presence of cases from other municipalities in Paraná (10.4%) and Paraguay (2.1%) highlights the importance of infection control measures (screening for resistant samples and geographic monitoring of these strains) that transcend geographic borders[4,22].

Regarding education, 24 (50%) individuals failed to provide such information during the registration process. Among the others, the majority had completed elementary school (20.8%), followed by completed secondary school (16.7%), incomplete elementary school (three cases – 6.25%), and incomplete secondary school (6.25%).

Underreporting and lack of data related to the lack of education information for half of the individuals are critical issues. They highlight the urgent need to improve the collection of demographic information in medical records, since the availability of detailed and reliable data is essential to guide the implementation of health promotion strategies at different levels, whether local, regional, national, or even international. Obtaining accurate information about patient profiles is essential for formulating health policies that meet the specific needs of each community, thus improving medical care and promoting health more efficiently and comprehensively[23-24.]

If we analyze the cases in which education data were available in this study, the majority of individuals had completed elementary education (20.8%). This highlights the importance of

targeting educational measures related to infection prevention for this group. However, this data may be corrupted by the lack of information provided in the registration of these patients.

Regarding health coverage, the Unified Health System (SUS) was the most common plan used among these patients (62.5%), followed by other health plans (33.3%) and private plans (4.2%).

The predominance of the Unified Health System (SUS) as a health plan among patients (62.5%) may indicate that the majority of those affected do not have the financial resources for private health plans and reinforces the need for investments in public health and improvement of the SUS care system to deal with antibiotic-resistant infections. In this context, the use of strategies for more appropriate and targeted treatments, together with cost reduction, becomes essential. By improving the treatment of antibiotic-resistant diseases in this system (SUS), we not only meet the needs of patients who depend on it, but also contribute to the efficiency of public resources and to containing the spread of bacterial resistance, which is a global threat to public health[25].

The origin of the samples was mainly concentrated in intensive care units (47.9%), followed by hospitalization wards (45.8%) and emergency entrance doors, that is, the Emergency Care and Emergency Room sectors (6.25%).

These findings are consistent with research conducted in different hospitals in Brazil, which also revealed high rates of positivity of microbial cultures of biological samples in critical areas, such as intensive care units[26-27]. The collection of samples primarily in intensive care units (47.9%) and inpatient wards (45.8%) highlights the importance of surveillance in critical hospital settings, where patients often have weakened immune systems and are at greater risk of infections[28].

Furthermore, the intense manipulation and use of invasive devices allows greater accessibility to infectious processes and the use of more potent medications, often unnecessarily, facilitating the selection of resistant microorganisms[13].

The results of this study reveal a worrying prevalence of infections caused by strains of *Klebsiella pneumoniae* producing the carbapenemase enzymes KPC and/or NDM in the municipality of Foz do Iguaçu, Paraná. These enzymes are known for their ability to inactivate or hydrolyze beta-lactam antibiotics, including carbapenems, which makes these infections challenging to treat[29-31].

Analysis of the resistance profile of the strains revealed that the KPC enzyme was the most predominant (60.4%), followed by NDM (27.1%)[32]. An epidemiological study conducted by Wang et al.[33] identified the prevalence of the KPC-2 gene in different regions, including South America, China, and the United States. Notably, the presence of co-resistance (12.5%) was observed less frequently in this study. However, in a parallel study carried out by Thomas et al.34 during the pandemic, lower co-resistance values were recorded for this same profile in several countries, such as Colombia (3.6%), Brazil (4.7%), and Uruguay (5.6%), with only six cases recorded in Paraguay. In contrast, Argentina showed notable positivity (96%) in this context.

These findings reveal the complexity of the spread of bacterial resistance and the geographic variation in co-resistance rates. Therefore, understanding these patterns is essential to guide the creation of control and treatment actions, especially in areas where co-resistance is more pronounced, as in the case of Argentina. Furthermore, these studies reinforce the importance of continuous epidemiological surveillance and collaborative research on a global scale to combat the growing threat of antibiotic-resistant infections, especially in areas with high human circulation, such as border areas.

Although Foz do Iguaçu (PR) is located in this region of great population mobility, where a migratory flow of 289,820 people was recorded in just one month (January/2024)[41] and receives tourists from all over the world, the KPC study continues to be an isolated phenomenon in this location. To date, there are no records of other studies addressing bacterial resistance in this city. However, based on the results of this study, it is suggested that resistance to carbapenems, mainly KPC, constitutes a threat to the Brazilian border with Paraguay and Argentina.

This scenario highlights the importance of early diagnosis of resistant strains, since inadequate management can facilitate the dissemination of plasmids containing resistance information to other microorganisms, which, in turn, can negatively impact therapy and the clinical outcome of patients.

Resistance to multiple antibiotics was a prominent feature of the strains, except for ceftazidime/avibactam (39.6%) and colistin (22.9%)[35]. These findings contrast with a previous study conducted in the State of Paraná by Pillonetto et al.[36], which reported lower rates of carbapenem resistance in a similar group of individuals. However, on the other hand, a study conducted by Silva et al.[37] identified a sensitivity profile more in line with the results obtained in this research, with sensitivity rates of 31.4% for colistin and 18.57% for ceftazidime/avibactam.

These findings emphasize the need to adopt rigorous infection control measures, accompanied by the encouragement of sound public policies, as well as the stimulation of the continuous development of new antimicrobial biomolecules. Medicinal plants, throughout history, have been shown to be valuable sources of bioactive compounds with antimicrobial properties. Exploring these natural resources not only expands our therapeutic arsenal but may also offer safer and more sustainable solutions to the growing bacterial resistance. Therefore, the convergence of these strategies is extremely important to deal with this problem, ensuring

the maintenance of the effectiveness of treatments and preserving public health comprehensively and sustainably.

Sensitivity to colistin and ceftazidime/tazobactam in some strains is encouraging, as it suggests the possibility of effective treatment in selected cases. However, the emergence of strains resistant to these antibiotics, as emphasized by Livermore[38], must be closely monitored, as it aims to anticipate outbreaks of resistant infections, guide the appropriate prescription of antibiotics, avoid inappropriate use, and contribute to the development of new drugs. Finally, monitoring is essential to maintain treatment effectiveness and protect public health.

The use of ceftazidime-avibactam has been shown to be a viable alternative to colistin in the treatment of carbapenemase-resistant strains, presenting a reduced risk of renal parenchymal damage due to its lower nephrotoxicity compared to colistin.[30].

According to data provided by the United States Food and Drug Administration (FDA), approval of new antibiotics has decreased by more than 50% in the last two decades. This highlights the importance of managing antimicrobial therapies judiciously to avoid irrational use and contain the development of resistance to these newer drugs[37].

In summary, this study highlights the prevalence and complexity of KPC and NDM infections in an emergency hospital in Foz do Iguaçu. These results highlight the need for comprehensive infection control strategies, epidemiological surveillance, and the development of new antibiotics to address bacterial resistance. Therefore, a deep understanding of patient profiles, including demographic factors, health conditions, and resistance patterns, allows for the creation of targeted preventive and therapeutic measures. Furthermore, the collection of epidemiological data contributes to active surveillance and early identification of outbreaks, enabling rapid and effective intervention.

Furthermore, the development of bioactive molecules, especially those derived from medicinal plants, opens up new perspectives in the fight against antibiotic-resistant infections.

These molecules offer an innovative and sustainable therapeutic approach that can complement the existing arsenal of drugs[4, 39-40].

CONCLUSION

The results of this study highlight the high prevalence of infections caused by *K. pneumoniae* producing KPC and/or NDM carbapenemases, with a predominance in male patients, elderly patients, and residents of Foz do Iguaçu. Most cases occurred in patients treated by the SUS and admitted to intensive care units, highlighting the impact of these infections in the hospital environment. The most frequent samples were rectal swabs and urine, highlighting the importance of epidemiological surveillance for tracking and controlling the spread of these multidrug-resistant pathogens. The resistance profile demonstrated a high degree of resistance to beta-lactams, aminoglycosides, and quinolones, with relatively preserved sensitivity only to colistin and ceftazidime/tazobactam. The identification of co-resistant strains reinforces the urgent need for rigorous infection control measures, rational use of antimicrobials, and the development of new therapeutic approaches to contain the spread of these clinically and epidemiologically relevant microorganisms.

Therefore, the implementation of socio-educational measures and greater public action through municipal health departments must be intensified in this region in order to strengthen epidemiological surveillance, contain the spread of multidrug-resistant pathogens, and reduce the impact of these infections on the population.

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EXTRATOS DE PLANTAS MEDICINAIS (*Psychotria viridis, Banisteropsis caapi* e *Terminalia catappa*) FRENTE A CEPAS RESISTENTES DE *KLEBSIELLA PNEUMONIAE* NA TRÍPLICE FRONTEIRA INTERNACIONAL (BRASIL X PARAGUAI X ARGENTINA)

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MEDICINAL PLANT EXTRACTS (*Psychotria viridis, Banisteropsis caapi* and *Terminalia catappa*) AGAINST RESISTANT STRAINS OF *Klebsiella pneumoniae* IN THE TRI-BORDER AREA (BRAZIL x PARAGUAY x ARGENTINA)

Antimicrobial potential of medicinal plants against resistant strains of *Klebsiella pneumoniae*

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MEDICINAL PLANT EXTRACTS (*Psychotria viridis, Banisteropsis caapi* and *Terminalia catappa*) AGAINST RESISTANT STRAINS OF *Klebsiella pneumoniae* IN THE TRI-BORDER AREA (BRAZIL x PARAGUAY x ARGENTINA)

ABSTRACT

Bacterial resistance is a problem that raises concerns due to its rapid growth, contributing to the inefficiency of next-generation antimicrobials. In this context, the objective of this study was to evaluate the activity of extracts from *Terminalia catappa, Banisteriopsis caapi*, and *Psychotria viridis* against strains *of Klebsiella pneumoniae* resistant to carbapenemases KPC and NDM. These samples were isolated from clinical samples from a Brazilian hospital located in a border region. Plant extracts were prepared, and the minimum inhibitory concentration (MIC) was determined using broth microdilution assays. The extract of *Psychotria viridis* showed antimicrobial activity, with MICs ranging from 2.5 mg/mL to 5 mg/mL for all tested strains. The extract of *Banisteriopsis caapi* exhibited MICs of 2.5 to 5 mg/mL, while *Terminalia catappa* had an MIC of 10 mg/mL. The combination of the extracts of *Psychotria viridis* and *Banisteriopsis caapi* achieved an MIC of 20 mg/mL. In summary, extracts of *Psychotria viridis* and *Banisteriopsis caapi* demonstrated significant bacteriostatic potential against resistant strains of *K. pneumoniae*, particularly *Psychotria viridis*. Although the combination of *Banisteriopsis caapi* and *Psychotria viridis* extracts did not exhibit synergism, their individual applications represent a promising approach, as highlighted by the high efficacy demonstrated in inactivating resistant strains. Future research should focus on identifying active compounds, their mechanisms of action, and the potential synergism with antimicrobials, aiming to develop new therapies against multidrug-resistant strains and to benefit border communities.

INTRODUCTION

The increase in infections caused by multidrug-resistant bacteria, such as *Klebsiella pneumoniae*, is a growing challenge for global public health due to the limitation of therapeutic options (Aguiar et al., 2023; WHO, 2022). This Gram-negative bacillus stands out for its ability to acquire and disseminate resistance genes, such as those encoding *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-beta-lactamase (NDM), conferring resistance to

carbapenems, antibiotics considered last-resort treatments, thus increasing morbidity and mortality rates as well as public health costs (Batista, 2020).

In the border regions between Brazil, Paraguay, and Argentina, the circulation of resistant strains may be exacerbated by intense population mobility and the integration of healthcare systems among these countries, creating a favorable environment for the dissemination of these pathogens (EClinicalMedicine 2021). Their rapid transmission in hospital and community settings, coupled with the multifactorial resistance of many pathogens, results in the emergence of multidrug-resistant strains or even strains resistant to all available antibiotics (pan-resistant), leading to an alarming shortage of therapeutic options (WHO, 2022). This highlights the urgent need for new antimicrobial agents, particularly bioactive compounds derived from natural resources.

Over time, plant extracts have been used in traditional medicine to treat various diseases, including bacterial infections, and scientific research has confirmed their therapeutic potential (Vaou et al., 2021). In Brazil, policies such as the National Program on Medicinal Plants and Phytotherapics (PNPMF) and the National Policy on Integrative and Complementary Practices (PNPIC) integrate phytotherapeutics into the Unified Health System (SUS). This promotes their safe use and regulation by the National Health Surveillance Agency (ANVISA). These initiatives enhance biodiversity, traditional knowledge, and contribute to cultural preservation, sustainable economic development, and environmental protection (Caccia-Bava et al., 2017; Carvalho et al., 2011; Palhares et al., 2020).

In this context, plants such as *Terminalia catappa, Banisteriopsis caapi, and Psychotria viridis* stand out due to their proven medicinal properties, making them the focus of significant scientific interest (Azevedo, 2018; Mwangi et al., 2024; Rossi et al., 2022).

Traditionally used to treat inflammation and liver diseases (Mwangi et al., 2024), *Terminalia catappa*, commonly known as the tropical almond tree, is found in tropical regions, mainly in coastal areas of northeastern, southern, and southeastern Brazil, as well as in Africa and Asia. Botanically, it is a medium to large tree whose leaves, fruits, and bark contain bioactive compounds such as tannins, flavonoids, and phenolic acids, with antioxidant, anti-inflammatory, and hepatoprotective properties, and can yield up to 8.75% in crude ethanolic extract (Terças et al., 2017).

Banisteriopsis caapi, also known as "mariri" or "jagube," is a vine native to the Amazon rainforest, mainly found in the northern and central-western regions of Brazil, in humid tropical forest areas. It is popularly recognized for its use in preparing *Ayahuasca* tea and contains

alkaloids such as harmine and tetrahydroharmine, which act as monoamine oxidase inhibitors (MAOIs) (Azevedo, 2018).

To date, many studies have explored its neuromodulatory properties. However, there is a hypothesis that the alkaloids in B. caapi may have the ability to combat certain Gram-positive (S. aureus) and Gram-negative (E. coli) pathogens (Gonçalves et al., 2023).

Psychotria viridis, commonly known as "chacrona," is a perennial shrub native to the Amazon, distributed in the northern and central-western regions of Brazil, especially in tropical forest areas. Its leaves contain N,N-Dimethyltryptamine (DMT), a potent psychedelic alkaloid that, in combination with the MAOI inhibitors of B. caapi, produces consciousness-expanding effects. According to Azevedo (2018), it can yield up to 13.47%. Although little studied, P. viridis contains molecules that may have antimicrobial activity in addition to its psychotherapeutic potential (Ruffell et al., 2023).

Thus, exploring the therapeutic potential of native plants such as *T. catappa, B. caapi, and P. viridis* may contribute to the development of new antimicrobial agents capable of acting against multidrug-resistant pathogens, like *K. pneumoniae*. In this context, the objective of this study was to evaluate the efficacy of hydroalcoholic extracts of these three plants against *K. pneumoniae* strains resistant to KPC and NDM carbapenemases, isolated from clinical samples in a Brazilian hospital located in a border region (Brazil x Paraguay x Argentina).

MATERIAL AND METHODS

Ethical aspects

This study was approved by the Research Ethics Committee (REC) of Universidade Paranaense – UNIPAR, under opinion no. 5.171.267. The bacterial strains analyzed were obtained during the clinical routine of the hospital where the research was conducted, as part of standard diagnostic procedures. It is noteworthy that these samples were not collected exclusively for research purposes but rather during routine examinations of hospitalized patients.

Sample and sampling

The samples in this study consisted of biological materials collected from individuals seeking medical care at a hospital due to various health disorders, such as urinary discomfort,

fever, muscle pain, and blood pressure changes. The study included blood, urine, rectal swabs, tracheal secretions, and tissue samples from patients hospitalized in a private hospital located in the border region between Brazil, Paraguay, and Argentina. These samples were obtained from hospitalized patients.

The sampling process included bacterial cultures isolated from these biological materials that tested positive for carbapenemase-producing Enterobacterales, specifically *Klebsiella pneumoniae* producing *Klebsiella pneumoniae* carbapenemase (KPC) or New Delhi metallo-β-lactamase (NDM), between December 2021 and December 2022. From the total number of samples collected during this period, 10 KPC strains, 10 NDM strains, and 5 co-resistant strains (KPC and NDM) were selected.

The identification of resistant strains was carried out by the hospital's laboratory service. Microbial culture and antimicrobial susceptibility testing were used for microbiological identification, while molecular identification was performed using real-time PCR (TaqMan).

Collection of plant material

Psychotria viridis and Banisteriopsis caapi extracts (Ayahuasca)

The fresh leaves of *Psychotria viridis* and the vine of *Banisteriopsis caapi* were collected in August 2020 at the Fogo Sagrado shamanic center in Mirassolândia, São Paulo (S20°36'49.14"; W49°26'44.2"). The collection was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, A6B0D96).

The samples were initially dried in an oven at 45 °C for five days, following adapted procedures according to Bussmann et al. (2010). After drying, the leaves and vine were ground. To obtain the extracts, an infusion was performed in boiling water at a ratio of 1L per 60g of dried material. After natural cooling of the infusions, the content was filtered to remove solids, followed by the addition of absolute ethanol at a 1:3 ratio. Then, the ethanol was removed by rotary evaporation. The final fraction obtained was subjected to lyophilization to ensure the preservation of the compounds (Bitwell et al., 2023).

After the procedure was finished, the extract was kept between 2 and 8 °C in a refrigerator until it was needed.

Terminalia catappa extract (Sete copas)

The fresh leaves of *Terminalia catappa* (Sete copas, specimen number 331) were collected in Umuarama, PR, Brazil (S23°45'52.4", W53°16'20.5") in October 2018, in the late afternoon. The collection was registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, ACCE9FA). The specimen was properly deposited in the Educational Herbarium of Universidade Paranaense (UNIPAR). After collection, the leaves were oven-dried, pulverized, and stored in paper bags in a dry and protected environment.

For the preparation of the hydroalcoholic extract of *T. catappa*, the plant material was macerated at room temperature for seven days using 70% ethanol in a 9:1 ratio (ethanol: powdered leaves). After the maceration period, the extracts were filtered and concentrated under reduced pressure using rotary evaporation, maintaining the temperature below 55°C. Finally, the extract was lyophilized at the Laboratory of Preclinical Research on Natural and Bioactive Products at Universidade Paranaense, following the procedures described by Brasil (2023).

Determination of antimicrobial activity

Broth microdilution - Minimum inhibitory concentration (MIC)

To determine the Minimum Inhibitory Concentration (MIC), a microdilution assay was conducted in BHI (Brain Heart Infusion) broth using a 96-well microplate. Initially, a bacterial suspension was prepared from previously cultured bacterial isolates, adjusted to a turbidity equivalent to the 0.5 McFarland standard, resulting in a concentration of approximately 1.5 x 10^8 colony forming units (CFU/mL). This suspension was subsequently diluted in a 1:10 ratio, reaching an approximate concentration of 1.5 x 10^7 CFU/mL (CLSI, 2019).

The tested extracts were used at a concentration of 40 mg/mL, with 200 µL distributed in the first well and a serial dilution performed (40mg/mL to 0,3125 mg/mL). For the combination of *P. viridis* and *B. caapi*, the extracts were mixed in a 1:1 ratio to obtain the initial concentration of 40 mg/mL.

In each well, 10 µL of the bacterial suspension were added. The microplate was then homogenized and incubated at 37°C for 24 hours.

After the incubation period, 10 µL of a 10% 2,3,5-triphenyltetrazolium chloride solution were added to each well for result development, with a new incubation for 60 minutes at 37°C.

The presence of bacterial growth was indicated by the pink coloration in the wells, as described by Moussa *et al*. (2013).

The isolated extracts and BHI broth were used as negative controls, while the BHI broth with inoculum was used as a positive control. The experiments were performed in triplicate, and the MIC was defined as the lowest concentration of the extracts in mg/mL capable of inhibiting microbial growth.

Statistical analysis

The data obtained were subjected to analysis of variance (ANOVA) to evaluate the existence of statistically significant differences between the experimental groups. Subsequently, the comparison between the means was performed using the Tukey test, ensuring robustness in the detection of specific variations between the treatments. In addition, the analysis of the combined extracts of P. viridis and B. caapi was performed using the Friedman test followed by Dunn's post-hoc test, due to the non-parametric nature of the data. The significance level adopted for all analyses was 5% ($p<0.05$), ensuring statistical rigor. And finally, the analyses were conducted using the statistical software GraphPad Prism version 8.4.3.

RESULTS

The plant extracts of *T. catappa, P. viridis, B. caapi*, and the combination of *P. viridis + B. caapi* were evaluated for their efficacy against resistant strains of *Klebsiella pneumoniae* KPC (*Klebsiella pneumoniae* carbapenemase), NDM (New Delhi metallo-β-lactamase), and the co-resistance of KPC+NDM. The results obtained can be seen in Table 1.

Regarding the efficacy of the different extracts tested for the treatment of infections caused by carbapenemase-producing strains of the KPC, NDM, and KPC+NDM types, the *P. viridis* extract demonstrated the best inhibitory activity, with the lowest minimum inhibitory concentration (MIC) for all tested strains, ranging between 2.5 mg/mL and 5 mg/mL. This low concentration indicates that this extract presents greater efficacy compared to the other tested extracts, such as *T. catappa* and *B. caapi*, which required higher concentrations (5-10 mg/mL). On the other hand, the combination of *P. viridis + B. caapi* exhibited the highest minimum inhibitory concentration (20 mg/mL), suggesting that the isolated extract of *P. viridis* is a

promising and more potent option for treating infections caused by KPC- and NDM-producing strains.

***Terminalia catappa* (Sete copas)**

The sete copas extracts demonstrated a minimum inhibitory concentration (MIC) of 10 mg/mL against all tested strains. This result demonstrated the same antimicrobial activity of this plant material; however, it requires a relatively high dose compared to synthetic antimicrobials used to treat infections caused by these microorganisms. Nevertheless, it remains within the expected range for many plant extracts to inhibit bacterial growth (Chassagne et al., 2020; Gonzalez-Pastor *et al.*, 2023).

***Banisteriopsis caapi* (Mariri)**

The *B. caapi* extracts exhibited an MIC of 5 mg/mL against most strains, including KPC1 to KPC8 and all NDM and KPC/NDM strains. However, the KPC9 strain was inhibited at a lower concentration of 2.5 mg/mL, which may indicate greater sensitivity to this plant extract.

***Psychotria viridis* (Chacrona)**

The *P. viridis* extracts exhibited variable MICs, with most strains being inhibited at 5 mg/mL, except for strains KPC1 to KPC7, which required only 2.5 mg/mL. However, strains such as KPC8, KPC9 and KPC10 required 5 mg/mL, suggesting variable resistance to the extract.

***P. viridis* + *B. caapi* combination**

The combination of *P. viridis* and *B. caapi* extracts resulted in an MIC of 20 mg/mL for all strains, except for KPC9, which showed an MIC of 10 mg/mL. Thus, an increase in MIC was observed in the combination of extracts compared to the individual extracts.

The assays were validated according to the results obtained through positive and negative controls at each stage. It is worth noting that synthetic antimicrobials were not used as controls due to the high resistance presented by some strains, which could introduce variations in the results and thus raise doubts about the validity of the tests.

Statistical analysis

Both the analysis of variance (ANOVA) and the Friedman test applied to the samples revealed a significant difference among the extract groups, indicating that at least one extract has a different MIC compared to the others. Tukey's and Dunn's post hoc tests identified these differences among specific groups, as shown in Table 2.

The results demonstrated that the *T. catappa* extract exhibited significantly lower antibacterial activity compared to the B. caapi and P. viridis extracts against both KPC and NDM-producing strains ($p < 0.0001$ for both comparisons). These findings suggest that the active compounds present in *B. caapi* and *P. viridis* have greater inhibitory potential against these carbapenemase variants. However, no statistically significant difference was observed between *B. caapi* and *P. viridis* in the same analyses ($p = 0.0923$ for KPC; $p = 0.4759$ for NDM), indicating comparable antibacterial efficacy between these two individual extracts.

In contrast, the combination of *B. caap*i and *P. viridis* extracts displayed an antagonistic effect, with significantly higher MIC values compared to the individual extracts. This reduction in efficacy was observed against both KPC and NDM strains ($p < 0.0001$), as well as against strains producing both carbapenemases simultaneously ($p = 0.0132$). Additionally, comparisons between the combination and the *T. catappa* extract revealed no statistically significant difference ($p > 0.9999$), further supporting the hypothesis that the combined use of the extracts offers no therapeutic advantage over the more effective individual extracts.

These findings point to a possible negative interaction between the bioactive compounds present in *B. caapi* and *P. viridis* when administered in combination, leading to a loss of the antibacterial activity previously observed when the extracts were used individually. This type of antagonism may be related to competition between secondary metabolites, altered bioavailability of active compounds, or negative modulation of antimicrobial action pathways.

In summary, the data demonstrate that although *B. caapi* and *P. viridis* extracts exhibit promising activity against carbapenemase-producing strains, their combination results in a significant reduction in efficacy, particularly against multidrug-resistant strains.

Discussion

Considering that the spread of multidrug-resistant bacteria has turned bacterial infections into one of the greatest global public health challenges (Lancet, 2022; WHO, 2022), the efficacy of many conventional antimicrobials has been compromised by the extraordinary adaptive capacity of pathogens, thus driving the search for new therapeutic alternatives (Aljeldah, 2022).

In this context, natural products, especially those derived from medicinal plants, have emerged as promising sources of bioactive compounds with antimicrobial potential, and the diversity of phytoconstituents such as flavonoids, terpenoids, and alkaloids ensures these substances have a broad spectrum of activities against different bacterial species, including those resistant to multiple antimicrobials. Additionally, the complex chemical matrix of medicinal plants may act synergistically, enhancing their antimicrobial effects, thereby minimizing the emergence of bacterial resistance (Gyawali and Ibrahim, 2014; Niu and Li, 2019; Ventola, 2015).

In this study, the results obtained from tests with extracts of *T. catappa, P. viridis, B. caapi*, and the combination of *P. viridis* + *B. caapi* against KPC and NDM strains showed significant variations in the antimicrobial activity of these plants.

The extract of *T. catappa* showed a MIC of 10 mg/mL against all tested strains. Considering that this extract contains a wealth of bioactive compounds, widely recognized, particularly in relation to antimicrobial properties, the result presented in this study further reinforces this statement, as a MIC with the same performance (10 mg/mL) was observed against all tested strains, highlighting the potential of this extract as a potencial alternative in the fight against clinically relevant pathogens.

Mbengui *et al.* (2013) reported a MIC of 16.67 mg/mL and a minimum bactericidal concentration (MBC) of 41.67 mg/mL for the ethanolic extract of *T. catappa*, indicating good efficacy for this purpose. According to Mbengui *et al.* (2013), the MICs ranged from 25 to 100 mg/mL for resistant strains, such as those producing extended-spectrum beta-lactamases (ESBL), demonstrating the extract's ability to inhibit the growth of beta-lactam-resistant strains.

Sowmya and Raveesha (2021) corroborated the findings of Mbengui *et al.* (2013) by observing MICs of 1.25 mg/mL for methanolic and acetone extracts of *T. catappa* in *K. pneumoniae* strains isolated from clinical samples at the Department of Microbiology of Mysuru Medical College in India.

Although the chemical composition of the *T. catappa* extract was not determined in the present study, this explanation is plausible to justify the differences observed. The phytochemical profile of medicinal plants can vary significantly depending on seasonal and environmental factors, which influence the biosynthesis of active compounds. Triterpenes, for example, tend to accumulate during autumn and winter due to lower precipitation and natural senescence, whereas flavonoids are more abundant in spring, correlating with leaf development and increased rainfall following dry periods (Zanatta et al., 2023). These phytochemical shifts could directly affect the antimicrobial efficacy of the extracts, as observed in previous studies such as that by Mbengui et al. (2013), which reported MIC values between 16.67 and 100 mg/mL for *T. catappa* against resistant strains.

To ensure consistent and viable results for *T. catappa* in clinical settings, it is essential that the methods for preparing and testing the extracts are standardized, especially when exploring the therapeutic potential of this plant against infections caused by multidrug-resistant strains. Such standardization becomes even more relevant considering that most current research on this plant material focuses on its antioxidant and anti-inflammatory properties, leaving significant gaps in understanding its efficacy against resistant pathogens, such as *K. pneumoniae* (Zanatta *et al*., 2023).

Moreover, the lack of studies evaluating *T. catappa* extract in clinical samples, especially in strains resistant to antimicrobials, reinforces the need for further scientific investigation in this area. Expanding research on the effects of *T. catappa* against multidrug-resistant bacteria could lead to new therapeutic approaches, offering promising alternatives in the fight against resistant infections and contributing to reducing the impact of bacterial infections on public health (Saharan *et al.,* 2022).

B. caapi extracts showed a MIC of 5 mg/mL against most strains, including KPC1 to KPC8, and all NDM and KPC/NDM strains. However, strains such as KPC9 exhibited a lower MIC of 2.5 mg/mL, suggesting greater sensitivity to this specific extract. The differences in MIC observed in the KPC9 strain can be explained by subtle genetic variations that make it more sensitive to the antimicrobial compounds present in *B. caapi* extract. These genetic variations, which could be point mutations in the genome, deletions, insertions, or variations in the copy number of specific genes affecting protein expression or systems related to antimicrobial resistance, may influence how KPC9 interacts with the active compounds, resulting in greater extract efficacy and, consequently, a lower MIC (Nascimento et al., 2000; Vaou et al., 2021).

Furthermore, KPC9 may have differences in its metabolic profile or the expression of resistance genes compared to other KPC strains. These metabolic differences or variations in gene expression may make it more susceptible to the bioactive compounds in *B. caapi* extract, and there could be synergistic interactions between the compounds in the extract that are particularly effective against the resistance mechanisms of KPC9, which would explain the lower MIC observed in this strain (Casanova and Costa, 2017).

Bussmann *et al*. (2010) conducted a study that demonstrated the efficacy of *B. caapi* extract against *E. coli* strains (ATCC 25922), showing an extremely low MIC of 0.0625 mg/mL. This result highlights the potent antimicrobial effect of *B. caapi* on *E. coli*, suggesting that the bioactive compounds in the plant may be highly effective against this bacterium.

The *P. viridis* extract exhibited inhibitory activity at a concentration of 5 mg/mL for most bacterial strains. However, strains KPC1 to KPC7, as well as NDM3 and NDM4, were more susceptible, showing inhibition at a lower concentration of 2.5 mg/mL. In contrast, strains such as KPC8, KPC9, and KPC10 required the full 5 mg/mL for effective inhibition, reflecting a higher degree of resistance. These findings indicate that the antibacterial activity of *P. viridis* varies among strains, likely due to differences in their pathogenicity or resistance mechanisms, suggesting that its bioactive compounds interact differently depending on the bacterial profile.

A study by Gonçalves *et al.* (2020) revealed that *P. viridis* extract by decoction showed a MIC of 10 mg/mL against several strains, including *E. faecalis* and *S. aureus*, but was less effective against *E. coli*, with a MIC greater than 10 mg/mL. On the other hand, *B. caapi* showed a MIC of 2.5 mg/mL against *L. monocytogenes* and *P. aeruginosa*, being particularly effective against *A. baumannii*, with a MIC of 0.625 mg/mL. However, the combination of these extracts showed reduced efficacy against other strains, suggesting that the interaction between the compounds does not always result in a synergistic effect. These findings reinforce the potential of the extracts, either individually or in combination, especially against resistant strains like *A. baumannii*.

The combination of *P. viridis* and *B. caapi* extracts in this study resulted in a MIC of 20 mg/mL for all tested strains, except KPC9, which showed a MIC of 10 mg/mL. This increase in MIC when the extracts were combined, compared to the individual extracts, suggests a possible antagonism of the active compounds. Therefore, it is important to consider that, despite the combination of the extracts, the efficacy was not superior and, in fact, a higher concentration was required to achieve bacterial growth inhibition.

One hypothesis to explain this reduction in antimicrobial potential is the possibility of antagonistic interactions between the bioactive compounds in the extracts. Such interactions can occur when the compounds interfere with each other's activity, resulting in a lower overall efficacy than when the extracts are used separately. Additionally, the combination of the extracts may alter the pH or solubility of the active compounds, which could compromise their bioavailability and, consequently, their ability to inhibit bacterial growth (Sharma *et al.,* 2020).

Moreover, this study sought to verify the antimicrobial potential of the extracts that make up Ayahuasca tea in the way it is consumed. Thus, the extraction method employed, the infusion in boiling water, although widely used for obtaining water-soluble compounds, may not have been the most suitable for efficiently extracting the alkaloids, since they require an acidic pH for their extraction, which are known for their antimicrobial potential. Therefore, the limitation in extracting these bioactive compounds may have directly impacted the observed antimicrobial activity, reducing their effectiveness against the tested strains (Santos *et al*., 2023).

Another plausible explanation would be competition for microbial targets, where the compounds in each extract compete for the same action sites within the microorganisms. This may result in a lower effective concentration of each active compound, leading to a weakened antimicrobial effect. These hypotheses highlight the complexity of interactions between natural compounds and the importance of further studies to fully understand the dynamics involved (Casanova and Costa, 2017).

No scientific study directly evaluating the effects of *P. viridis* or *B. caapi*, separately or together, on *Klebsiella pneumoniae* strains was found. Most research involving these plants focuses on their use in traditional medicine, particularly in the context of Ayahuasca, a beverage that combines these two species and is widely studied for its psychoactive properties rather than its antimicrobial effects (Azevedo, 2018; Gonçalves *et al.*, 2023; Rossi *et al.*, 2022; Ruffell *et al.*, 2023).

Thus, with its favorable environment for cultivating and managing these plants, along with its widespread availability across various regions of the country, Brazil stands out as a strategic hub for the development of research focused on investigating biological agents with antimicrobial potential, paving the way for important advances in this field.

Primary research on these plants revolves around their neuropharmacological activities due to the presence of alkaloids such as DMT (*P. viridis*) and β-carbolines such as harmine, harmaline, and tetrahydroharmine (*B. caapi*). These compounds are the main focus of studies

investigating the impact of Ayahuasca on the central nervous system and its potential therapeutic uses, including the treatment of mental health disorders (Politi *et al*., 2022).

Given that antimicrobial resistance is a serious issue and increasingly concerning due to its rapid spread, studies that use clinical samples, such as those conducted in this work, are of fundamental importance, as they accurately reflect the reality found in hospital environments. Furthermore, these studies allow an understanding of the challenges in therapeutic management and the difficulties encountered in controlling infections caused by resistant pathogens, such as KPC and NDM carbapenemases, especially in areas of international triple-border regions. In these areas, where the movement of people and microorganisms is intense, the obstacles to containing the spread of multidrug-resistant bacterial strains become even more evident, emphasizing the need for innovative and effective approaches for managing these infections.

CONCLUSION

The extract of *Psychotria viridis* stood out as the most promising option, exhibiting the lowest minimum inhibitory concentration (MIC) among the tested extracts, suggesting greater efficacy in inhibiting these resistant strains. In contrast, the combination of *P. viridis* and *B. caapi* resulted in the highest MIC, indicating a possible interaction that reduced the expected antimicrobial activity. Statistical analysis confirmed significant differences between the extracts, emphasizing that antimicrobial efficacy is influenced by the type of extract used.

The *T. catappa* extract demonstrated an intermediate potential, with a MIC higher than that of *P. viridis* but lower than that of the combination of *P. viridis* and *B. caapi*. This reinforces the need for further studies to optimize extraction methods and assess potential interactions between the bioactive compounds present in these extracts.

These findings highlight the potential of *P. viridis* as a possible candidate for the development of new therapeutic strategies against infections caused by multidrug-resistant pathogens, while also underscoring the need for further studies to optimize extraction methods and assess potential interactions between the bioactive compounds present in these extracts.

Therefore, future investigations should focus on identifying specific active compounds and elucidating the antimicrobial mechanisms of action, as well as exploring the possibility of modifying the extracts to increase their efficacy, such as investigating whether there is positive

synergy between plant extracts and conventional antimicrobials against strains producing resistance mechanisms like carbapenemases.

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AUTHORSHIP CONTRIBUTION STATEMENT

1. Aline Cristiane Cechinel Assing Batista – Conceptualization, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization.
2. Adriane Cordeiro Trevisani - Conceptualization, Investigation, Writing - Review & Editing.
3. Mariana Dalmagro - Conceptualization, Investigation.
4. Mariana Pinc - Conceptualization, Investigation.
5. Jorge Luis Maria Ruiz - Formal analysis, Investigation.
6. Karina Sakumoto - Formal analysis, Investigation.
7. Odair Alberton - Investigation.
8. Andre Giarola Boscarato - Investigation.
9. Zilda Cristiani Gazim - Supervision, Project administration.
10. Lidiane Nunes Barbosa - Supervision, Project administration.
11. Daniela Dib Gonçalves - Supervision, Project administration, Conceptualization, Writing - Review & Editing.

STATEMENT

No competing financial interests exist.

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Table 1. Minimum inhibitory concentration (MIC) (mg/mL) of *Terminalia catappa, Banisteropsis caapi,* and *Psychotria viridis* extracts and their combination, tested against *Klebsiella pneumoniae* strains producing carbapenemase (KPC), New Delhi metallo-beta-lactamase (NDM), and strains with both resistance mechanisms (KPC + NDM), isolated from biological samples collected from hospitalized patients in an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

Strains	***Terminalia catappa*** (mg/mL)	***Banisteropsis caapi*** (mg/mL)	***Psychotria viridis*** (mg/mL)	***P. viridis* + *B. caapi*** (mg/mL)
KPC1	10	5	2,5	20
KPC2	10	5	2,5	20
KPC3	10	5	2,5	20
KPC4	10	5	2,5	20
KPC5	10	5	2,5	20
KPC6	10	5	2,5	20
KPC7	10	5	2,5	20
KPC8	10	5	5	20
KPC9	10	2,5	5	10
KPC10	10	5	5	20
NDM1	10	5	5	20
NDM2	10	5	5	20
NDM3	10	5	2,5	20
NDM4	10	5	2,5	20
NDM5	10	5	5	20
NDM6	10	5	5	20
NDM7	10	5	5	20
NDM8	10	5	5	20
NDM9	10	5	5	20
NDM10	10	5	5	20
K/N1	10	5	5	20
K/N2	10	5	5	20
K/N3	10	5	5	20
K/N4	10	5	5	20

K/N5	10	5	5	20

Legend: KPC (*Klebsiella pneumoniae* carbapenemase); NDM (New-Delhi-metallo-beta-lactamase); K/N (*Klebsiella pneumoniae* carbapenemase + New-Delhi-metallo-beta-lactamase).

Table 2. Analysis of variance (ANOVA) of *Terminalia catappa, Banisteropsis caapi*, and *Psychotria viridis* extracts and their combination, tested against *Klebsiella pneumoniae* strains producing carbapenemase (KPC), New Delhi metallo-beta-lactamase (NDM), and strains with both resistances (KPC + NDM), isolated from biological samples collected from hospitalized patients in an emergency hospital in Foz do Iguaçu (Paraná, Brazil) from December 2021 to December 2022.

Extracts	**p-valor**		
	KPC	**NDM**	**KPC/ NDM**
Statistical test	**(Tukey)**	**(Tukey)**	**(Dunn)**
T. catappa vs. B. caapi	**<0,0001**	**<0,0001**	0,3972
T. catappa vs. P. viridis	**<0,0001**	**<0,0001**	0,3972
T. catappa vs. B. caapi + P. viridis	**<0,0001**	**<0,0001**	>0,9999
B. caapi vs. P. viridis	0,0923	0,4759	>0,9999
B. caapi vs. B. caapi + P. viridis	**<0,0001**	**<0,0001**	**0,0132**
P. viridis vs. B. caapi + P. viridis	**<0,0001**	**<0,0001**	**0,0132**

3. CONCLUSÃO

1. Este estudo revelou uma prevalência de infecções por *Klebsiella pneumoniae* produtora de carbapenemases KPC e/ou NDM, predominantemente em pacientes masculinos, idosos e residentes em Foz do Iguaçu. A maioria dos casos ocorreu em pacientes do SUS internados em UTIs, frisando o impacto dessas infecções no ambiente hospitalar. As amostras mais frequentes foram swabs retais e urina, destacando a importância da vigilância epidemiológica para o rastreamento e controle da disseminação desses patógenos multirresistentes.

   O perfil de resistência mostrou altos níveis de resistência a betalactâmicos, aminoglicosídeos e quinolonas, com sensibilidade preservada apenas para colistina e ceftazidima/tazobactam. A identificação de cepas com co-resistência reforça a necessidade de controle rigoroso de infecções, uso racional de antimicrobianos e novas estratégias terapêuticas.

   Assim, é fundamental intensificar medidas socioeducativas e a atuação do poder público, especialmente das secretarias municipais de saúde, para fortalecer a vigilância epidemiológica, conter a disseminação de microrganismos multirresistentes e reduzir o impacto dessas infecções na população.

2. O extrato de *Psychotria viridis* demonstrou a maior eficácia antimicrobiana entre os extratos testados, apresentando a menor concentração inibitória mínima (CIM) e destacando-se como um potencial agente contra cepas resistentes. Em contraste, a combinação com *Banisteriopsis caapi* resultou na maior CIM, sugerindo uma interação que reduziu sua atividade antimicrobiana. A análise estatística confirmou diferenças significativas entre os extratos, evidenciando a influência do tipo de extração na eficácia antimicrobiana.

   O extrato de *T. catappa* demonstrou um potencial intermediário, com uma CIM maior que *P. viridis* mas inferior que a combinação de *P. viridis* e *B. caapi*.

   Esses achados reforçam o potencial de *P. viridis* para o desenvolvimento de novas terapias contra patógenos multirresistentes e destacam a necessidade de estudos adicionais para otimizar métodos de extração e investigar interações entre compostos bioativos. Futuras pesquisas devem focar na identificação dos compostos ativos, no esclarecimento dos mecanismos de ação e na modulação dos extratos para

aumentar sua eficácia. Além disso, deve-se explorar a sinergia entre os extratos vegetais e antimicrobianos convencionais, especialmente contra cepas produtoras de mecanismos de resistência, como as carbapenemases.

4. APÊNDICES

APÊNDICE A–Relatório de coleta de dados das amostras positivas.

RELATÓRIO DE COLETA DE DADOS DAS AMOSTRAS POSITIVAS

Sexo: ( ) M ( ) F

Idade:_____

Tipo de amostra: _____

Período de internação: _____

Setor de internamento:_____

Convênio: ( ) Particular ( ) SUS ( ) outros

Patologia: _____

Desfecho clínico: _____

Local de residência: _____

Nível de escolaridade: _____

5 ANEXOS

ANEXO 1 -Comitê de Ética em Pesquisa Envolvendo Seres Humanos (CEPEH)

UNIVERSIDADE PARANAENSE - UNIPAR

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Potencial antibacteriano de plantas medicinais em cepas de Klebsiella pneumoniae KPC e NDM.

Pesquisador: Daniela Dib Gonçalves

Área Temática:

Versão: 2

CAAE: 53134621.7.0000.0109

Instituição Proponente: Universidade Paranaense

Patrocinador Principal: ASSOCIACAO PARANAENSE DE ENSINO E CULTURA

DADOS DO PARECER

Número do Parecer: 5.171.267

Apresentação do Projeto:

Este projeto será submetido à autorização pela instituição a qual serão coletados os dados - Hospital Ministro Costa Cavalcanti Fundação de Saúde Itaiguapy, através de um termo de consentimento para a disponibilização dos isolados. Além de submissão no Comitê de Ética em Pesquisa (CEP) / Comissão Nacional de Ética em Pesquisa (CONEP) / Plataforma Brasil, para obtenção de parecer favorável ao desenvolvimento da pesquisa. Serão utilizadas 129 cepas bacterianas com perfil de resistência aos carbapenêmicos oriundas de amostras de sangue, urina, fezes, swabs retais e quaisquer secreções (traqueal, lavado broncoalveolar e abscessos) destinadas à realização dos exames laboratoriais de rotina de pacientes

internados que derem entrada no laboratório de análises clínicas do Hospital Ministro Costa Cavalcanti (HMCC) no período de novembro de 2021 a novembro de 2022. Serão selecionadas uma amostra por paciente que apresentar resultado positivo para Klebsiella pneumoniae KPC, NDM ou ambas as resistências, durante o período acima citado, a fim de evitar vários clones de um mesmo indivíduo e ampliar o escopo de pesquisa. Após

separadas, serão selecionadas 10 unidades de cada cepa apresentando resistência (KPC, NDM e KPC+NDM). Serão incluídos na seleção, amostras de pacientes adultos (18 anos), com resultado de cultura positiva há pelo menos 7 dias de hospitalização, ambos os sexos e em uso de antibioticoterapia invasiva. Para o diagnóstico laboratorial humano será realizado as técnicas microbiológicas descritas neste projeto e já estabelecidas como padrões junto ao respectivo

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hospital que posteriormente entregará a coordenadora deste projeto os resultados microbiológicos e a respectiva amostra biológica para que seja possível a realização dos testes microbiológicos com as duas plantas propostas. Também será
entregue os dados das fichas (com o devido anonimato) a coordenadora do projeto para tabulação e tratamento estatístico. Identificação microbiana A identificação das cepas ficará a cargo do laboratório de análises clínicas do HMCC e será realizada por meio de identificação bioquímica e concentração inibitória mínima estimada em automação através do equipamento Vitek 2 (BioMerieux®), onde serão selecionados isolados de Klebsiella pneumoniae que apresentaram perfil de resistência para os antibióticos da classe das penicilinas, cefalosporinas, carbapenens e monobactâmicos. Para a identificação serão utilizados os cartões GN (BioMerieux®) e para o antibiograma os cartões 238 (BioMerieux®) para amostras urinárias e cartões 239 (BioMerieux®) nas demais amostras. Os parâmetros utilizados para seleção de triagem de resistência das cepas analisadas serão MIC 2µg/ mL para meropenem/ imipinem e MIC 1 µg/ mL para ertapenem. Após selecionadas as cepas, elas serão isoladas em TSB com glicerol a 20% e armazenadas em freezer -80°C.ExtratosExtrato etanólico de AyahuascaÓleo essencial de Euphorbia tirucalli.

Objetivo da Pesquisa:
Objetivo Primário:

Avaliar a ação antibacteriana de extratos das plantas Euphorbia tirucalli, Banisteriopsis caapi e Psychotria viridis em cepas de Klebsiella pneumoniae do tipo KPC e NDM oriundas de um Hospital de urgência em Foz do Iguaçu, PR.

Objetivo Secundário:
- Objetivos específicos Selecionar 129 isolados de Klebsiella pneumoniae com perfil de resistência aos carbapenêmicos KPC e NDM entre novembro de 2021 e novembro de 2022;
- Organizar as 129 cepas em 3 grupos (KPC, NDM e KPC/NDM), e selecionar 10 exemplares
de cada grupo (30 cepas) para realização das análises microbiológicas com os extratos;
- Obter o extrato de folhas de P. viridis e E. tirucalli e de caules de B. caapi
utilizando o etanol como solvente extrator;
- Submeter os extratos a testes de atividade antimicrobiana frente as cepas selecionadas; - Mensurar os halos de inibição e MIC gerados após cultura com as cepas selecionadas;
- Sequenciar as cepas selecionadas através do método de Sanger;
- Realizar ações de educação em saúde para disseminação de conhecimento para a comunidade,

onde será elaborado uma cartilha informativa e entrevista sobre o tema de resistência antimicrobiana na região de fronteira.

Avaliação dos Riscos e Benefícios:
Riscos: Nenhum

Benefícios:
- Avaliar um elevado número de cepas positivas para carbapenemases do tipo KPC e NDM quanto à ação antibacteriana das plantas Euphorbia tirucalli, Banisteriopsis caapi e Psychotria viridis.
- Verificar a ação in vitro da produção de bioativos secundários das mesmas, frente aos microrganismos isolados, através da visualização dos halos de inibição pela técnica de disco-difusão.
- Contribuir para a descoberta de novas terapias antimicrobianas para o tratamento de doenças provocadas por estes isolados, bem como ampliar o banco de dados para pesquisas avançadas nessa área de atuação.
- Ademais, a realização desse tipo de pesquisa em área de intensa mobilização populacional fronteiriça, permite ampliar o escopo de pesquisa sobre o tema abordado, permitindo não apenas a testagem em cepas características da região, mas a possibilidade de abordar cepas importadas de outras regiões e desta forma, permitindo a implementação de ações de melhoria abrangentes.

Comentários e Considerações sobre a Pesquisa:
Este projeto de pós graduação é uma pesquisa aplicada de procedimento experimental. Neste sentido, a pesquisa se apresenta de forma conclusiva, possui relevância e pode ser executada, uma vez que os pesquisadores contemplaram todos os requisitos éticos para a sua realização.

Considerações sobre os Termos de apresentação obrigatória:
Folha de rosto: Aprovado
Termo de anuência institucional: Aprovado
Carta de dispensa do Termo de Consentimento livre e esclarecido - TCLE: Aprovado

Recomendações:
Parecer:
Caro pesquisador, seu projeto apresenta relevância, e para que ele possa ser executada, é necessário que os pesquisadores sigam os preceitos éticos conforme descrito abaixo:

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De acordo com a Resolução 466/12 – III - Dos aspectos éticos da pesquisa envolvendo seres humanos – III.1 – A eticidade da pesquisa implica em:

i) Prever procedimentos que assegurem a confidencialidade e a privacidade, a proteção da imagem e a não estigmatização dos participantes da pesquisa, garantindo a não utilização das informações em prejuízo das pessoas e/ou das comunidades, inclusive em termos de autoestima, de prestígio e/ou de aspectos econômico-financeiros;

Atenciosamente.

Conclusões ou Pendências e Lista de Inadequações:

Prezado pesquisador, vosso projeto foi aprovado sem restrições.

Atenciosamente.

Considerações Finais a critério do CEP:

Aprovado sem restrições.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1835057.pdf	27/11/2021 07:12:25		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	DispensaTCLE.pdf	27/11/2021 07:10:51	Daniela Dib Gonçalves	Aceito
Outros	TAIcarimbado.pdf	09/11/2021 08:38:21	Daniela Dib Gonçalves	Aceito
Projeto Detalhado / Brochura Investigador	Projeto29112021.pdf	29/09/2021 16:22:39	Daniela Dib Gonçalves	Aceito
Folha de Rosto	folhaDeRosto.pdf	29/09/2021 16:06:22	Daniela Dib Gonçalves	Aceito

Situação do Parecer:

Aprovado

Endereço: Praça Mascarenhas de Moraes, 8482
Bairro: Umuarama **CEP:** 87.502-210
UF: PR **Município:** UMUARAMA
Telefone: (44)3621-2849 **Fax:** (44)9127-7860 **E-mail:** cepeh@unipar.br

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Continuação do Parecer: 5.171.267

Necessita Apreciação da CONEP:
Não

UMUARAMA, 16 de Dezembro de 2021

Assinado por:
Ana Carolina Soares Fraga Zaze
(Coordenador(a))

Endereço: Praça Mascarenhas de Moraes, 8482
Bairro: Umuarama **CEP:** 87.502-210
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ANEXO 2 - Normas da Revista Ciência & Saúde Coletiva

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INSTRUÇÕES PARA COLABORADORES

Ciência & Saúde Coletiva publica debates, análises e resultados de investigações sobre um tema específico considerado relevante para a saúde coletiva; e artigos de discussão e análise do estado da arte da área e das subáreas, mesmo que não versem sobre o assunto do tema central. A revista, de periodicidade mensal, tem como propósitos enfrentar os desafios, buscar a consolidação e promover uma permanente atualização das tendências de pensamento e das práticas na saúde coletiva, em diálogo com a agenda contemporânea da Ciência & Tecnologia.

Os artigos serão avaliados através da Revisão de pares, de acordo com as diretrizes internacionais para a área da ciência.

> *Política de Acesso Aberto - Ciência & Saúde Coletiva é publicada sob o modelo de acesso aberto e é, portanto, livre para qualquer pessoa a ler em download, e para copiar e divulgar para fins educacionais. Nossa política editorial segue a comunicação de pesquisa no modus operandi de Ciência Aberta.*

A Revista Ciência & Saúde Coletiva aceita artigos em *preprints* de bases de dados nacionais e internacionais reconhecidas academicamente.

Ao acessar o sistema ScholarOne evite utilizar o tradutor automático do navegador de internet.

No momento que você apresenta seu artigo, é importante estar atento ao que constitui um *preprint* e como você pode proceder para se integrar nesta primeira etapa da Ciência Aberta. O *preprint* disponibiliza artigos e outras comunicações científicas de forma imediata ou paralela à sua avaliação e validação pelos periódicos. Desta forma, acelera a comunicação dos resultados de pesquisas, garante autoria intelectual, e permite que o autor receba comentários que contribuam para melhorar seu trabalho, antes de submetê-lo a algum periódico. Embora o artigo possa ficar apenas no repositório de *preprints* (caso o autor não queira mandá-lo para um periódico), as revistas continuam exercendo as funções fundamentais de validação, preservação e disseminação das pesquisas. Portanto:

(1) São aceitáveis as submissões de preprints dos seguintes servidores: SciELO Preprints, arXiv, bioRxiv e medRxiv ou outro servidor confiável. A aceitação de Preprints de outros servidores será analisada pelos editores do periódico.
Se aprovado, ele receberá um *DOI* que garante sua divulgação internacional imediata.
(2) Concomitantemente, caso você queira, pode submetê-lo à Revista Ciência & Saúde Coletiva. Os dois processos são compatíveis.

(3)Você pode optar por apresentar o artigo apenas à Revista Ciência & Saúde Coletiva. A submissão a repositório *preprint* não é obrigatória.

Desde janeiro de 2021, está sendo cobrada uma **taxa de submissão** de **R$ 100,00** (cem reais) **para artigos nacionais** e **US$ 25,00** (vinte e cinco dólares) **para artigos internacionais**. O valor não será devolvido em caso de recusa do material. Para pagamento da taxa de submissão, acesse o site da Revista (https://cienciaesaudecoletiva.com.br/). Este apoio dos autores é indispensável para

financiar o custeio da Revista, viabilizando a publicação com acesso universal dos leitores. **Não é cobrada taxa de publicação**. Caso o artigo vá para avaliação e receba o parecer *Minor Revision* (Pequena revisão) ou *Major Revision* (Grande Revisão) não é necessário pagar a taxa novamente quando enviar a revisão com as correções solicitadas. Somente os artigos de chamada pública com recursos próprios estão isentos de pagamento de taxa de submissão.

Recomendações para a submissão de artigos

Notas sobre a Política Editorial

A Revista Ciência & Saúde Coletiva reafirma sua missão de **veicular artigos originais, que tragam novidade e proporcionem avanço no conhecimento da área de saúde coletiva**. Qualquer texto que caiba nesse escopo é e será sempre bem-vindo, dentro dos critérios descritos a seguir:

(1) O artigo não deve tratar apenas de questões de interesse local ou situar-se somente no plano descritivo.

(2) Na sua introdução, o autor precisa deixar claro o caráter inédito da contribuição que seu artigo traz. Também é altamente recomendado que, na carta ao editor, o autor explicite, de forma detalhada, porque seu artigo constitui uma novidade e em que ele contribui para o avanço do conhecimento.

(3) As discussões dos dados devem apresentar uma análise que, ao mesmo tempo, valorize especificidade dos achados de pesquisa ou da revisão, e coloque esses achados em diálogo com a literatura nacional e internacional.

(4) O artigo qualitativo precisa apresentar, de forma explícita, análises e interpretações ancoradas em alguma teoria ou reflexão teórica que promova diálogo das Ciências Sociais e Humanas com a Saúde Coletiva. Exige-se também que o texto valorize o conhecimento nacional e internacional.

(5) Quanto aos artigos de cunho quantitativo, a revista prioriza os de base populacional e provenientes de amostragem aleatória. Não se encaixam na linha editorial: os que apresentam amostras de conveniência, pequenas ou apenas descritivas; ou análises sem fundamento teórico e discussões e interpretações superficiais.

(6) As revisões não devem apenas sumarizar o atual estado da arte, mas precisam interpretar as evidências disponíveis e produzir uma síntese que contribua para o avanço do conhecimento. Assim, a nossa orientação é publicar somente revisões

de alta relevância, abrangência, originalidade e consistência teórica e metodológica, que de fato tragam novos conhecimentos ao campo da Saúde Coletiva.

(7) A versão final do artigo aprovado será publicada com o nome do editor ou editores-chefes responsáveis pelo processo de avaliação do manuscrito.

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Nota importante - Dado o exponencial aumento da demanda à Revista, todos os artigos passam por uma triagem inicial, realizada pelos editores-chefes. Sua decisão sobre o aceite ou não é baseada nas prioridades citadas e no mérito do manuscrito quanto à originalidade, pertinência da análise estatística ou qualitativa, adequação dos métodos e riqueza interpretativa da discussão. Levando em conta tais critérios, apenas uma pequena proporção dos originais, atualmente, é encaminhada para revisores e recebe parecer detalhado.

A revista *C&SC* adota as "Normas para apresentação de artigos propostos para publicação em revistas médicas", Vancouver, da Comissão Internacional de Editores de Revistas Médicas, cuja versão para o português encontra-se publicada na *Rev Port Clin Geral* 1997; 14:159-174. O documento está disponível em vários sítios na World Wide Web, como por exemplo, www.icmje.org ou www.apmcg.pt/document/71479/450062.pdf. **Recomenda-se aos autores a sua leitura atenta. Consultem os exemplos no final das Normas**.

Seções da publicação

Editorial: de responsabilidade dos editores chefes ou dos editores convidados, deve ter no máximo 4.000 caracteres com espaço.

Artigos Temáticos: devem trazer resultados de pesquisas de natureza empírica, experimental, conceitual e de revisões sobre o assunto em pauta. Os textos de pesquisa não deverão ultrapassar os 40.000 caracteres. Os artigos temáticos são selecionados da seguinte forma: por chamada pública, convite ou por coletânea de artigos já aprovados. **Na modalidade chamada pública, os manuscritos deverão ser encaminhados somente para o e-mail da chamada específica daquele número temático (não serão aceitos manuscritos postados na plataforma de submissão da Revista).**

Artigos de Temas Livres: devem ser de interesse para a saúde coletiva por livre apresentação dos autores através da página da revista em fluxo contínuo. Devem ter as mesmas características dos artigos temáticos: máximo de 40.000 caracteres com espaço, resultarem de pesquisa e apresentarem análises e avaliações de tendências teórico-metodológicas e conceituais da área.

Artigos de Revisão: devem ser textos baseados exclusivamente em fontes secundárias, submetidas a métodos de análises já teoricamente consagrados, podendo alcançar até o máximo de 45.000 caracteres com espaço.

Opinião: texto que expresse posição qualificada de um ou vários autores ou entrevistas realizadas com especialistas no assunto em debate na revista; deve ter, no máximo, 20.000 caracteres com espaço.

Resenhas: análise crítica de livros relacionados ao campo temático da saúde coletiva, publicados nos últimos dois anos, cujo texto não deve ultrapassar 10.000 caracteres com espaço. O autor deve atribuir um título para a resenha no campo título resumido (*running head*) quando fizer a submissão. Os autores da resenha devem incluir no

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início do texto a referência completa do livro. As referências citadas ao longo do texto devem seguir as mesmas regras dos artigos. No momento da submissão da resenha os autores devem inserir em anexo no sistema uma reprodução, em alta definição da capa do livro em formato jpeg. Não é necessário resumo, abstract e resumen.
Cartas: com apreciações e sugestões a respeito do que é publicado em números anteriores da revista (máximo de 4.000 caracteres com espaço). Não é necessário resumo, abstract e resumen.

Observação: Em artigos temáticos, temas livres, revisão e opinião, o limite máximo de caracteres leva em conta os espaços e inclui da palavra introdução e vai até a última referência bibliográfica.

O resumo(português) /abstract (inglês)/resumen (espanhol) com no máximo 1400 caracteres com espaço cada (a contagem inclui a palavra – "resumo"/"abstract"/"resumen" até a última "palavra-chave"/"keyword"/"palabra clave").

Apresentação de manuscritos

1. Os originais podem ser escritos em português, espanhol, francês e inglês. Os artigos obrigatoriamente deverão ter título e resumo em português, inglês e espanhol. Os textos em português devem ter título, resumo e palavras-chave na língua original, em inglês e em espanhol. Os textos em espanhol devem ter título, resumo e palavras- chave na língua original, em português e em inglês. Os textos em inglês devem ter título, resumo e palavras-chave na língua original, em português e em espanhol. Os textos em francês devem ter título, resumo e palavras-chave na língua original, em português e em inglês. **Não serão aceitas notas de pé-de-página no final das páginas dos artigos.**

2. Os textos têm de ser digitados em espaço duplo, na fonte Times New Roman, no corpo 12, margens de 2,5 cm, formato Word (de preferência na extensão .docx) e encaminhadosapenas pelo endereço eletrônico (http://mc04.manuscriptcentral.com/csc-scielo) segundo as orientações do site.

3. Os artigos publicados serão de propriedade da revista *C&SC*, ficando proibida a reprodução total ou parcial em qualquer meio de divulgação, impressa ou eletrônica, sem a prévia autorização dos editores-chefes da Revista. A publicação secundária deve indicar a fonte da publicação original.

4. Os artigos submetidos à C&SC não podem ser propostos simultaneamente para outros periódicos.

5. As questões éticas referentes às publicações de pesquisa com seres humanos são de inteira responsabilidade dos autores e devem estar em conformidade com os princípios contidos na Declaração de Helsinque da Associação Médica Mundial (1964, reformulada em 1975,1983, 1989, 1989, 1996 e 2000).

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6. Os artigos devem ser encaminhados com as autorizações para reproduzir material publicado anteriormente, para usar ilustrações que possam identificar pessoas e para transferir direitos de autor e outros documentos.

7. Os conceitos e opiniões expressos nos artigos, bem como a exatidão e a procedência das citações são de exclusiva responsabilidade dos autores.

8. Os textos são em geral (mas não necessariamente) divididos em seções com os **Título, Resumo, Introdução, Métodos, Resultados e Discussão**, às vezes, sendo necessária a inclusão de subtítulos em algumas seções. Os títulos e subtítulos das seções não devem conter numeração progressiva e sim recursos gráficos como caixa alta, recuo na margem ou outros.

9. O título deve ter curto: 120 caracteres com espaço. O resumo/abstract/resumen, com no máximo 1.400 caracteres com espaço (incluindo a palavra resumo até a última palavra-chave)e precisa explicitar **o objeto**, **os objetivos**, **a metodologia**, **a abordagem teórica, os resultados** e **as conclusões.** Logo abaixo do resumo os autores devem indicar até no máximo, cinco (5) palavras-chave/keywords/palabras- clave. É fundamental ter clareza e objetividade na redação do resumo, pois assim o fazendo, o autor contribuirá para o interesse do leitor. Já clareza dos descritores contribuirá para a múltipla indexação do artigo.
As palavras-chave em português, inglês e espanhol devem constar obrigatoriamente no DeCS/MeSH. (http://www.ncbi.nlm.nih.gov/mesh/e http://decs.bvs.br/).

10. É obrigatória a inclusão do *Open Researcher and Contributor ID* (ORCID) no momento de submissão do artigo. Para criar um ID ORCID acesse: http://orcid.org/content/initiative10. Na submissão dos artigos na plataforma da Revista, é válido que apenas um autor tenha o registro no ORCID. Mas quando o artigo for aprovado para publicação no SciELO, **todos os autores** devem ter o registro no ORCID. Para se registrar no ORCID, entre no site (https://orcid.org/) e para inserir o ORCID no ScholarOne (plataforma de submissão), acesse o site (https://mc04.manuscriptcentral.com/csc-scielo), e atualize seu cadastro.

11. Em caso de usar inteligência artificial nos seus manuscritos, o autor deve mencionar esse fato, obrigatoriamente, dizendo ao final do campo dedicado à metodologia, em que etapa do artigo ela foi empregada.

12. Os manuscritos aprovados devem vir acompanhados de uma declaração de disponibilidade dos dados usados e gerados na pesquisa subjacentes aos textos. O modelo será enviado junto aos modelos das declarções solicitadas.

Autoria

1. As pessoas designadas como autores devem ter participado na elaboração dos artigos de modo que possam assumir publicamente a responsabilidade pelo seu conteúdo. A qualificação como autor deve pressupor: a) a concepção e o delineamento ou a análise e interpretação dos dados, b) redação do artigo ou a sua revisão crítica, e c) aprovação da versão a ser publicada.

2. O limite de autores por artigo é de oito autores, se exceder esse limite, os demais terão seus nomes incluídos nos agradecimentos. Para artigos com mais autores que fazem parte de um

grupo de pesquisa ou em outros casos excepcionais, é necessária autorização dos editores. Exceções serão discutidas pelo Corpo Editorial.

3. Em nenhum arquivo inserido, deverá constar identificação de autores do manuscrito, exceto no arquivo "Title page" (Página de título).

Nomenclaturas

1. Devem ser observadas rigidamente as regras de nomenclatura de saúde pública/saúde coletiva, assim como abreviaturas e convenções adotadas em disciplinas especializadas. Devem ser evitadas abreviaturas no título e no resumo.
2. A designação completa à qual se refere uma abreviatura deve preceder a primeira ocorrência desta no texto, a menos que se trate de uma unidade de medida padrão.

Ilustrações e Escalas

1. O material ilustrativo da revista *C&SC* compreende tabela (elementos demonstrativos como números, medidas, percentagens, etc.), quadro (elementos demonstrativos com informações textuais), gráficos (demonstração esquemática de um fato e suas variações), figura (demonstração esquemática de informações por meio de mapas, diagramas, fluxogramas, como também por meio de desenhos ou fotografias). Nas edições da revista que forem impressas, todo esse material será na cor preta e cores cinza para diferenciações.

2. O número de material ilustrativo deve ser de, **no máximo, cinco por artigo (com limite de até duas laudas cada)**, salvo exceções referentes a artigos de sistematização de áreas específicas do campo temático. Nesse caso os autores devem negociar com os editores-chefes.

3. Todo o material ilustrativo deve ser numerado consecutivamente em algarismos arábicos, com suas respectivas legendas e fontes, e a cada um deve ser atribuído um breve título. Todas as ilustrações devem ser citadas no texto.

4. Tabelas e quadros devem ser confeccionados no programa Word ou Excel e enviados com título e fonte. OBS: No link do IBGE (http://biblioteca.ibge.gov.br/visualizacao/livros/liv23907.pdf) estão as orientações para confeccionar as tabelas. Devem estar configurados em linhas e colunas, sem espaços extras, e sem recursos de "quebra de página". Cada dado deve ser inserido em uma célula separada. Importante: tabelas e quadros devem apresentar informações sucintas. As tabelas e quadros podem ter no máximo 15 cm de largura X 18 cm de altura e não devem ultrapassar duas páginas (no formato A4, com espaço simples e letra em tamanho 9).

5. Gráficos e figuras podem ser confeccionados no programa Excel, Word ou PPT. O autor deve enviar o arquivo no programa original, separado do texto, em formato editável (que permite o recurso "copiar e colar") e também em pdf ou jpeg, TONS DE CINZA ou coloridos. Gráficos gerados em programas de imagem devem ser enviados em jpeg, TONS DE CINZA ou coloridos, resolução mínima de 200 dpi e tamanho máximo de 20cm de altura x 15 cm de largura. As ilustrações coloridas só serão publicadas na versão online. Quando houver impressão da Revista, as ilustrações serão todas em TONS DE CINZA sem exceção. É importante que a imagem

original esteja com boa qualidade, pois não adianta aumentar a resolução se o original estiver comprometido. Gráficos e figuras também devem ser enviados com título e fonte. As figuras e gráficos têm que estar no máximo em uma página (no formato A4, com 15 cm de largura x 20cm de altura, letra no tamanho 9).

6. Arquivos de figuras como mapas ou fotos devem ser salvos no (ou exportados para o) formato JPEG, TIF ou PDF. Em qualquer dos casos, deve-se gerar e salvar o material na maior resolução (300 ou mais DPI) e maior tamanho possíveis (dentro do limite de 21cm de altura x 15 cm de largura). Se houver texto no interior da figura, deve ser formatado em fonte Times New Roman, corpo 9. Fonte e legenda devem ser enviadas também em formato editável que permita o recurso "copiar/colar". Esse tipo de figura também deve ser enviado com título e fonte.

7. Os autores que utilizam escalas em seus trabalhos devem informar explicitamente na carta de submissão de seus artigos, se elas são de domínio público ou se têm permissão para o uso.

Agradecimentos

1. Quando existirem, devem ser colocados antes das referências bibliográficas.
2. Os autores são responsáveis pela obtenção de autorização escrita das pessoas nomeadas nos agradecimentos, dado que os leitores podem inferir que tais pessoas subscrevem os dados e as conclusões.
3. O agradecimento ao apoio técnico deve estar em parágrafo diferente dos outros tipos de contribuição.

Financiamento

RC&SC atende à Portaria N^{0} 206 do ano de 2018 do Ministério da Educação/Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Gabinete sobre obrigatoriedade de citação da CAPES para os trabalhos produzidos ou publicados, em qualquer mídia, que decorram de atividades financiadas, integral ou parcialmente, pela CAPES. Esses trabalhos científicos devem identificar a fonte de financiamento através da utilização do código 001 para todos os financiamentos recebidos.

Referências

1. As referências devem ser numeradas de forma consecutiva de acordo com a ordem em que forem sendo citadas no texto. No caso de as referências serem de mais de dois autores, no corpo do texto deve ser citado apenas o nome do primeiro autor seguido da expressão *et al*. Exemplo: Minayo *et al.*[3]

2. Devem ser identificadas por números arábicos sobrescritos, conforme exemplos abaixo: ex. 1: "Outro indicador analisado foi o de maturidade do PSF" [11] (p.38). ex. 2: "Como alerta Maria Adélia de Souza [4], a cidade..."

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As referências citadas somente nos quadros e figuras devem ser numeradas a partir do número da última referência citada no texto.

3. As referências citadas devem ser listadas ao final do artigo, em ordem numérica, seguindo as normas gerais dos *Requisitos uniformes para manuscritos apresentados a periódicos biomédicos* (http://www.nlm.nih.gov/bsd/uniform_requirements.html).
4. Os nomes das revistas devem ser abreviados de acordo com o estilo usado no Index Medicus (https://www.ncbi.nlm.nih.gov/nlmcatalog/journals)
5. O nome de pessoa, cidades e países devem ser citados na língua original da publicação.

Exemplos de como citar referências

Artigos em periódicos

1. Artigo padrão (**incluir todos os autores sem utilizar a expressão** *et al*.). Se for usar o *et al.* deve ser colocado no caso de ter mais de 25 autores no artigo.

Pelegrini MLM, Castro JD, Drachler ML. Eqüidade na alocação de recursos para a saúde: a experiência no Rio Grande do Sul, Brasil. *Cien Saude Colet* 2005; 10(2):275- 286.

Maximiano AA, Fernandes RO, Nunes FP, Assis MP, Matos RV, Barbosa CGS, Oliveira- Filho EC. Utilização de drogas veterinárias, agrotóxicos e afins em ambientes hídricos: demandas, regulamentação e considerações sobre riscos à saúde humana e ambiental. *Cien Saude Colet* 2005; 10(2):483-491.

Andrade FMD, Machado ÍE, Freitas MIF, Souza MFM, Malta DC. Patterns of abuse of elderly people in Brazil: analysis of notifications. *Cad Saúde Publica* 2023; 39(1):e00075722.

2. Instituição como autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. *Med J Aust* 1996; 164(5):282-284.

3. Sem indicação de autoria

Cancer in South Africa [editorial]. *S Afr Med J* 1994; 84(2):15.

4. Número com suplemento

Duarte MFS. Maturação física: uma revisão de literatura, com especial atenção à criança brasileira. *Cad Saude Publica* 1993; 9(Supl.1):71-84.

5. Indicação do tipo de texto, se necessário

Enzensberger W, Fischer PA. Metronome in Parkinson's disease [carta]. *Lancet* 1996; 347(9011):1337.

Livros e outras monografias

1. Indivíduo como autor

Cecchetto FR. *Violência, cultura e poder.* Rio de Janeiro: FGV; 2004.
Minayo MCS. Pesquisa qualitativa. Ciên Saúde Colet 2025; 29(6):e19792023.

2. Organizador ou compilador como autor
Bosi MLM, Mercado FJ, organizadores. *Pesquisa qualitativa de serviços de saúde.* Petrópolis: Vozes; 2004.

3. Instituição como autor
Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA). *Controle de plantas aquáticas por meio de agrotóxicos e afins.* Brasília: DILIQ/IBAMA; 2001.

4. Capítulo de livro
Sarcinelli PN. A exposição de crianças e adolescentes a agrotóxicos. In: Peres F, Moreira JC, organizadores. *É veneno ou é remédio*. Agrotóxicos, saúde e ambiente. Rio de Janeiro: Fiocruz; 2003. p. 43-58.

5. Resumo em Anais de congressos
Kimura J, Shibasaki H, organizadores. Recent advances in clinical neurophysiology. *Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology;* 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

6. Trabalhos completos publicados em eventos científicos
Coates V, Correa MM. Características de 462 adolescentes grávidas em São Paulo. In: *Anais do V Congresso Brasileiro de adolescência*; 1993; Belo Horizonte. p. 581-582.

7. Dissertação e tese
Carvalho GCM. *O financiamento público federal do Sistema Único de Saúde 1988-2001* [tese]. São Paulo: Faculdade de Saúde Pública; 2002.

Gomes WA. *Adolescência, desenvolvimento puberal e sexualidade:* nível de informação de adolescentes e professores das escolas municipais de Feira de Santana – BA [dissertação]. Feira de Santana (BA): Universidade Estadual de Feira de Santana; 2001.

Outros trabalhos publicados

8. Artigo de jornal

Novas técnicas de reprodução assistida possibilitam a maternidade após os 40 anos. *Jornal do Brasil;* 2004 Jan 31; p. 12
Lee G. Hospitalizations tied to ozone pollution: study estimates 50,000 admissions annually. *The Washington Post* 1996 Jun 21; Sect. A:3 (col. 5).

9. Material audiovisual
HIV+/AIDS: the facts and the future [videocassette]. St. Louis (MO): Mosby-Year Book; 1995.

10. Documentos legais

Brasil. Lei nº 8.080 de 19 de setembro de 1990. Dispõe sobre as condições para a promoção, proteção e recuperação da saúde, a organização e o funcionamento dos serviços correspondentes e dá outras providências. *Diário Oficial da União* 1990; 19 set.

Material no prelo ou não publicado

Leshner AI. Molecular mechanisms of cocaine addiction. *N Engl J Med*. In press 1996.
Cronemberg S, Santos DVV, Ramos LFF, Oliveira ACM, Maestrini HA, Calixto N. Trabeculectomia com mitomicina C em pacientes com glaucoma congênito refratário. *Arq Bras Oftalmol.* No prelo 2004.

Material eletrônico

11. Artigo em formato eletrônico
Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* [serial on the Internet].1995 jan-mar [cited 1996 Jun 5];1(1):[about 24 p.]. Available from: http://www.cdc.gov/ncidod/EID/eid.htm

Lucena AR, Velasco e Cruz AA, Cavalcante R. Estudo epidemiológico do tracoma em comunidade da Chapada do Araripe – PE – Brasil. *Arq Bras Oftalmol* [periódico na Internet]. 2004 mar-abr [acessado 2004 Jul 12];67(2): [cerca de 4 p.]. Disponível em: http://www.abonet.com.br/abo/672/197-200.pdf

12. Monografia em formato eletrônico
CDI, clinical dermatology illustrated [CD-ROM]. Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2ª ed. Version 2.0. San Diego: CMEA; 1995.

13. Programa de computador
Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

ANEXO 3 - Normas da Revista Vector – Borne and Zoonotic Diseases

Manuscript Submission Guidelines and Policies for *Vector-Borne and Zoonotic Diseases*

Last updated 8/12/2024 8:42:30 PM

Journal Information

- **Manuscript Submission Site: https://mc.manuscriptcentral.com/vbz**
- **Editorial Office Contact:** shiggs@k-state.edu Including pre-submission inquiries.
- **Support Contact:** prosupport@liebertpub.com
- **Journal Model:** Hybrid (Open Access available)
- **Identity Transparency:** Single anonymous
- **File formatting requirement stage:** On Submission
- **Associated Fees:** Click to see Publication Costs
- Average time to initial decision: 50+ days

About the Journal

Vector-Borne and Zoonotic Diseases is a peer-reviewed journal that includes articles on diseases transmitted to humans by mosquitoes, ticks, and other arthropod vectors or directly by other animals. The Journal emphasizes the following topics: ecology, entomology, microbiology, immunology, and wildlife biology, as well as other subjects relevant to the transmission of infectious diseases to humans directly from the environment or through vectors.

A primary goal of this peer-reviewed journal is the integration of field, laboratory, and clinical sciences for the purpose of establishing causal relationships between clinical medicine and the environment that will lead to improved understanding of this group of neglected but important diseases and contribute to their successful management and prevention. The Journal develops and encourages publication standards to foster effective and reliable data collection, analysis, interpretation, and communication.

Manuscript Types and Guidelines

Original Research Articles	• 3,000-word limit • Structured abstract of no more than 300 words • Maximum of six (6) tables and/or figures
Review Articles	*Reviews are summaries of developments in the field.* • 5,000-word limit • Structured abstract of no more than 300 words • Maximum of eight (8) tables and/or figures
Short Communications	• 1,000-word limit • Structured abstract of no more than 150 words

	• May include one (1) figure OR table • Maximum of ten (10) references
Editorials and Commentaries	*Editorials and commentaries may be on any issue relevant to the field, but must be brief and appropriately documented by data.* • 1,000-word limit • An abstract is not required • No tables or figures
Letters to the Editor	• 500-word limit • May include one (1) figure OR table • Reference citations are identical in style to those of full original articles, but should not exceed five (5).
Protocol	The Protocol manuscript type is dedicated to supporting the awareness and publication of operating procedures for methodologies that reinforce key advances in the field. The step-by-step protocol provided in a Protocol Article is intended to establish peer-reviewed methodologies and enable technical improvements for specialists and non-specialists. The Protocol Article submission should describe a method that has already been used to produce results in a peer-reviewed original research article and should describe a technological or methodological update or advancement when compared to the "state-of-the art" methodology. **Every submitted Protocol Article must provide data and compare the new process to existing processes or identify gaps in prior related protocol publications.** • 4,000-word limit • 350-word structured abstract • Composition: Introduction, Method, Experiment, Results, and Discussion • 10 figures maximum • 6 tables maximum

Word limits do NOT pertain to the abstract, disclosure statements, author contribution statements, funding information, acknowledgments, tables, figure legends, or references.

References

Vector-Borne and Zoonotic Diseases uses Mary Ann Liebert's **Harvard** style reference format. Templates are available in **Zotero** and through the CSL Style Repository. An **Endnote template** is also available.

Liebert Harvard Style (Name/Date)

- Reference list: Include a complete list of all cited references in alphabetical (last) name/date order
- NOTE: References with the same author list and publication year should be distinguished by using lower case letter after the year of publication (Ex: Jones, 2020a; Jones, 2020b), both in text and in the reference list.
- Citation within text: At the point of citation and in parentheses, include only author surname(s) followed by a comma, then year of publication (Ex: One author: Jones, 2020; Two authors: Jones and Smith, 2021; Three or more authors: Jones et al, 2022).
- Journal titles should follow the abbreviation style of PubMed/Medline.

- Include among the references any articles that have been accepted but have not yet published; identify the name of publication and add "In Press." If the reference has been published online, provide the DOI number in place of the page range.

Style Examples for Reference List:

Type of Reference	Punctuation and Order of Elements in Reference List
Journal article with 1-3 authors	Wang Q, Nambiar K, Wilson JM. Isolating natural adeno-associated viruses from primate tissues with a high-fidelity polymerase. Hum Gene Ther 2021;32(23-24):1439-1449; doi: 10.1089/hum.2021.055 [insert article-specific DOI if available].
Journal article with more than 3 authors	Pfister EL, DiNardo N, Mondo E, et al. Artificial miRNAs reduce human mutant Huntington throughout the striatum in a transgenic sheep model of Huntington's disease. Hum Gene Ther 2018;29(6):663–673; doi: 10.1089/hum.2017.199 [insert article-specific DOI if available].
Edited Book	Herzog RW, Zolotukhin S, (eds). A Guide to Human Gene Therapy. World Scientific Publishing Co. Pte. Ltd.: Singapore; 2010.
Chapter in an Edited Book	Nicklin SA, Baker AH. Adenoviral Vectors. In: A Guide to Human Gene Therapy. (Herzog RW, Zolotukhin S. eds.) World Scientific Publishing Co. Pte. Ltd.: Singapore, 2010; pp. 21-36.
Authored Book	Isaacson W. The Code Breaker: Jennifer Doudna, Gene Editing, and the Future of the Human Race. Simon & Schuster: New York, NY; 2021.
Website	Last name, first/middle initial(s) of author(s) [if available]. U.S. Food and Drug Administration. What is Gene Therapy? Silver Spring, MD; 2018. Available from: **https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/what-gene-therapy** [Last accessed: month/date/year].
Personal communications	References that are unpublished (ie: personal communications, emails, letters) are not to be included in the reference list. Instead, insert “Personal communication; [name], date” parenthetically at the point of citation within text.
Using previously published images or tables as a reference	Reused/adapted images, tables, or any published material must be officially cited as a reference in the reference list, and the author(s) of the submitted work must obtain written permission from the copyright holder. Verbal approvals are not acceptable. Any fees associated with the reuse or adaptation of any material is the sole responsibility of the author(s).

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All manuscripts must be submitted through the journal's ScholarOne Manuscripts site.

General Manuscript Submission Guidelines and Policies for Mary Ann Liebert Journals

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Submission Preparation

All manuscripts must be prepared in accordance with the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (icmje.org). Please consult your specific journal's requirements for additional information.

All Mary Ann Liebert, Inc. journals follow the standards, guidelines, and best practices set forth by the Committee on Publication Ethics (COPE; publicationethics.org), the International Committee of Journal Medical Editors (ICJME; www.icmje.org), the World Medical Association (WMA); www.wma.net), and the American Medical Association (www.ama-assn.org).

Mary Ann Liebert, Inc. recommends that submissions follow standard relevant reporting guidelines. Please consult The Equator Network for more information.

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There is no obligation to use either the free or paid service. No editorial, review, nor any other decisions will be dependent on its use.

All manuscripts must be submitted through the journal's ScholarOne Manuscripts site. Please refer to the individual journal's instructions for more information and to access the service.

Manuscript Formatting

Please check your journal's requirements for file formatting. Many journals require formatting compliance only on revision; however, unless stated, the file formatting should comply with the following requirements on submission.

Manuscript Files

The main text file, figure legends, and tables should be prepared in Microsoft Word. Some journals may accept LaTex. Please consult your individual journal instructions for guidance.

File Naming

- All file names should be in English and contain only alphanumeric characters.
- **Do not include spaces, symbols, special characters, dashes, dots, or underscores.**
- Title each file with the type of content contained in the file (e.g., manuscript.doc, tables.doc, FigureLegends.doc, Fig1.tif, SupplementalData.pdf, etc.).

Figures

- Submission of high resolution .TIFF or .EPS figure files is preferred. Please upload as individual files.
- Cite figures consecutively in text within parentheses.
- Images should not reveal the name of a patient or a manufacturer.
- Note: Figures that will not be reproduced in color must be readable and interpretable in black and white.

Figure Legends

- A legend should be provided for each supplied figure.
- All legends should be numbered consecutively.
- Figure legends may be included at the end of the main text file or uploaded as a separate, double-spaced Word file.
- In each legend, provide explanations for any abbreviations or symbols that appear in the figure.
- If the figure is taken from a copyrighted publication, permission must be secured by the author(s) and supplied at the time of submission with appropriate credit listed in the legend. Permissions and associated fees are the responsibility of the author.

Tables

- Tables may be included after the references at the end of the main text file, or uploaded as a single, separate Word file. All tables should be editable.
- Provide a title for each supplied table.
- Cite tables sequentially in text within parentheses.
- Explain abbreviations used in the body of the table in footnotes using superscript letters, not symbols.
- If a table is taken from a copyrighted publication, permission must be secured by the author(s) and supplied at the time of submission with appropriate credit listed in the legend. Permissions and associated fees are the responsibility of the author.

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- Supplemental files should be uploaded as individual files. Most text, photo, graphic, and video formats are accepted. Ensure that patient identities are not revealed.
- Supplemental Information will not be copyedited or typeset; it will be posted online as supplied.
- For journals that publish accepted versions of papers prior to copyediting and typesetting, supplemental files will not be posted with the paper until after production has been completed.

Manuscript Structure

Specific journal requirements will vary, however the general order of elements in each manuscript should be

- Title page* with full manuscript title, all contributing authors' names and affiliations, a short running title, a denotation of the corresponding author, and a list of 4-6 keywords/search terms,
- Abstract,
- Main text without embedded figures or tables and with appropriate section headings, if applicable. Most research papers should be organized as follows: Introduction, Materials and Methods, Results, Discussion, and Conclusions.
- Acknowledgments,
- Authorship confirmation/contribution statement (CRediT format is preferred)
- Author(s') disclosure (Conflict of Interest) statement(s), even when not applicable,
- Funding statement, even when not applicable,
- References,
- Tables included in the text or as a separate document,
- Figure legends at the end of the main text or in a separate Word file,
- Figures uploaded as individual high-resolution files,
- Supplemental files uploaded as individual files.

*Double-blinded journals require a separate title page with the title, all contributing authors' names and affiliations, a denotation of the corresponding author, author acknowledgements, disclosures, and related identifying information.

Your individual journal may require

- An Institutional Review Board (IRB) approval (or waiver) statement and statement of patient consent as a separate paragraph after the methods section,
- Other relevant ethics attestations (see icmje.org for further guidance),
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- Specific abstract and content sections, depending on manuscript type,
- Word count limits, tables/figure limits, and reference format requirements.

Please note that paragaphs should be no longer than 15 lines once typeset.

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Upon manuscript submission, the submitting agent will have an opportunity to enter funding/grant information. If funding information is entered correctly, the publisher will deposit the funding acknowledgements from the article as part of the standard metadata to Funder Registry. The entered information should include funder names, funder IDs (if available), and associated grant numbers. Special care should be taken when entering this information to ensure total accuracy. Funding information must also be provided within the manuscript.

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Authorship Confirmation/Contribution Statement

An authorship contribution statement must be included with the manuscript. We strongly recommend that the authorship contribution statement follow the CRediT Taxonomy guidelines. (https://credit.niso.org/)

- Conceptualization (Ideas; formulation or evolution of overarching research goals and aims.)

- Data curation (Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.)
- Formal analysis (Application of statistical, mathematical, computational, or other formal techniques to analyze or synthesize study data.)
- Funding acquisition (Acquisition of the financial support for the project leading to this publication.)
- Investigation (Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection.)
- Methodology (Development or design of methodology; creation of models.)
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- Software (Programming, software development; designing computer programs; implementation of the computer code and supporting algorithms; testing of existing code components.)
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- Visualization (Preparation, creation and/or presentation of the published work, specifically visualization/data presentation.)
- Writing – original draft (Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation).)
- Writing – review & editing (Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre- or post-publication stages.)>

Example

Author 1: review and editing (equal). **Author 2**: Conceptualization (lead); writing – original draft (lead); formal analysis (lead); writing – review and editing (equal). **Author 3**: Software (lead); writing – review and editing (equal). **Author 4**: Methodology (lead); writing – review and editing (equal). **Author 5**: Conceptualization (supporting); Writing – original draft (supporting); Writing – review and editing (equal).

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16-Sep-2025

Dear Dr. Gonçalves:

Your manuscript entitled "MEDICINAL PLANT EXTRACTS (Psychotria viridis, Banisteropsis caapi and Terminalia catappa) AGAINST RESISTANT STRAINS OF Klebsiella pneumoniae IN THE TRI-BORDER AREA (BRAZIL x PARAGUAY x ARGENTINA)" has been successfully submitted online and is presently being considered for publication in Vector-Borne and Zoonotic Diseases.

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Title
CARBAPENEMASES: RESISTANCE PROFILE IN NA INTERNATIONAL BORDER HOSPITAL (Brazil X Paraguay X Argentina) CARBAPENEMASES

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