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STHEFANY DA CUNHA DIAS

Complexos de Paládio(II) e Platina(II) ligados a β-dicetonas como estratégia de controle *Campylobacter jejuni* na indústria avícola

Uberlândia
2026

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Tese apresentada ao Programa de Pós-Graduação em Ciências Veterinárias, da Faculdade de Medicina Veterinária, da Universidade Federal de Uberlândia, como exigência parcial para obtenção do título de Doutora em Ciências Veterinárias.

Área de concentração: Saúde Animal

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Dedico este trabalho aos meus pais e à minha irmã, que com amor, apoio e confiança sempre acreditaram nos meus sonhos. Cada conquista minha também pertence a vocês.

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“Entrega o teu caminho ao Senhor; confia nele, e ele tudo fará.” Salmos 37:5

RESUMO

Campylobacter jejuni é o principal agente etiológico responsável pela gastroenterite bacteriana em todo o mundo. A campilobacteriose está frequentemente associada ao consumo de alimentos contaminados, especialmente carne de frango. Fatores como a capacidade de colonizar diferentes ambientes, formar biofilmes e apresentar crescente resistência a antibióticos são um desafio para o controle desse patógeno na cadeia produtiva de alimentos e para a saúde pública. Nesse contexto, a busca por novos agentes antimicrobianos tem se intensificado, com destaque para compostos metálicos com potencial atividade biológica. Complexos metálicos contendo ligantes β-dicetonas têm despertado interesse devido à sua estabilidade estrutural, versatilidade química e potencial de interação com sistemas biológicos. Esta tese foi estruturada em três capítulos, sendo o primeiro dedicado às considerações gerais e ao referencial teórico dos temas abordados nos capítulos subsequentes. O segundo capítulo teve como objetivo avaliar a eficácia de complexos metálicos de paládio ligados a β-dicetonatos e dimetilsulfóxido (DMSO), de fórmula [$Pd^{2+}Cl_2$(4,4,4-trifluoro-1-(2-tienil)-1,3-butanodiona)(DMSO)] (PdDMSOTTA) e [$Pd^{2+}Cl_2$(4,4,4-trifluoro-1-fenil-1,3-butanodiona)(DMSO)] (PdDMSOBTA). O terceiro capítulo teve como objetivo investigar a ação de complexos de platina ligados a β-dicetonas, de fórmula [$Pt^{2+}Cl_2$(4,4,4-trifluoro-1-(2-tienil)-1,3-butanodiona)(DMSO)] (PtDMSOTTA) e [$Pt^{2+}Cl_2$(4,4,4-trifluoro-1-fenil-1,3-butanodiona)(DMSO)] (PtDMSOBTA). Os testes foram conduzidos em seis cepas de *C. jejuni* resistentes à eritromicina e à ciprofloxacina, avaliadas nas formas planctônica e séssil. A atividade bactericida dos complexos foi determinada por meio da concentração bactericida mínima (CBM). Todas as cepas foram classificadas pelo índice de formação de biofilme (IFB) em não formadoras, fracas, médias e fortes formadoras de biofilme; enquanto a atividade antibiofilme foi avaliada por ensaios de controle de biofilmes maduros mediante a determinação da redução do IFB. A ultraestrutura dos biofilmes foi analisada por microscopia eletrônica de varredura, o perfil metabolômico por cromatografia gasosa acoplada à espectrometria de massas (GC–MS) e a citotoxicidade em células Caco-2 diferenciadas pelo ensaio de redução da resazurina. Os testes apresentados no capítulo dois apresentam os resultados dos complexos de paládio coordenados a β-dicetonas. O complexo PdDMSOBTA apresentou maior atividade bactericida contra células planctônicas, com CBM média de 15,36 μg/mL, eliminando todas as cepas em concentrações < 50 μg/mL. Em biofilmes, o PdDMSOBTA promoveu eliminação completa em concentrações < 400 μg/mL e reduziu significativamente a biomassa aderida, com diminuição média de aproximadamente 75% no índice de formação de biofilme. A análise metabolômica revelou alterações em vias relacionadas ao metabolismo de aminoácidos, purinas e resposta ao estresse celular. A viabilidade das células Caco-2 permaneceu acima de 80–90% após exposição aos compostos, indicando baixa citotoxicidade. No capítulo três foi investigado o potencial antimicrobiano de complexos β-dicetonatos de platina(II), PtDMSOBTA e PtDMSOTTA, contra as mesmas seis cepas resistentes de *C. jejuni*. O complexo PtDMSOBTA apresentou maior atividade bactericida na forma planctônica, com CBM média de 12,76 μg/mL, enquanto o PtDMSOTTA apresentou menor eficácia. Em biofilmes, o PtDMSOBTA eliminou todos os isolados em concentrações < 200 μg/mL e reduziu significativamente ($p=0,0075$) a biomassa do biofilme, enquanto o PtDMSOTTA apresentou efeito moderado. A microscopia eletrônica de varredura revelou desorganização estrutural dos biofilmes e redução da matriz extracelular. A análise metabolômica identificou alterações em vias relacionadas ao metabolismo de aminoácidos, equilíbrio redox e do triptofano. Ambos os complexos apresentaram baixa citotoxicidade, com viabilidade celular entre 80 e 100%. O estudo permitiu concluir que complexos β-dicetonatos

de paládio(II) e platina(II), especialmente PdDMSOBTA e PtDMSOBTA, contendo o ligante 4,4,4-trifluoro-1-fenil-1,3-butanodiona, apresentam expressiva atividade antimicrobiana contra *C. jejuni* nas formas planctônica e séssil. Para células planctônicas, concentrações de até 100 μg/mL mostraram-se suficientes para eliminar o microrganismo, possivelmente em decorrência de alterações metabólicas associadas à indução de estresse celular. Além disso, os compostos apresentaram baixa citotoxicidade em células eucarióticas, indicando um perfil inicial de segurança biológica. Em relação à forma séssil, foram necessárias concentrações mais elevadas para o controle dos biofilmes. Estudos adicionais de citotoxicidade ainda são necessários para confirmar a segurança nessas condições. De modo geral, os resultados obtidos destacam o potencial desses complexos metálicos como alternativas promissoras para o controle de *C. jejuni*, especialmente em estratégias voltadas à redução da contaminação em ambientes relacionados à produção e ao processamento de alimentos.

Palavras-chave: Avicultura; Biofilmes; Citotoxicidade; Metabolômica; Resistência Bacteriana.

ABSTRACT

Campylobacter jejuni is the main etiological agent responsible for bacterial gastroenteritis worldwide. *Campylobacter*iosis is frequently associated with the consumption of contaminated foods, especially chicken meat. Factors such as the ability to colonize different environments, form biofilms, and exhibit increasing resistance to antibiotics represent a major challenge for controlling this pathogen in the food production chain and for public health. In this context, the search for new antimicrobial agents has intensified, with particular emphasis on metal-based compounds with potential biological activity. Metal complexes containing β-diketone ligands have attracted interest due to their structural stability, chemical versatility, and potential to interact with biological systems. This thesis was structured into three chapters. The first chapter is dedicated to general considerations and the theoretical background of the topics addressed in the subsequent chapters. The second chapter aimed to evaluate the efficacy of palladium metal complexes bound to β-diketonates and dimethyl sulfoxide (DMSO), with the formulas [$Pd^{2+}Cl_2$(4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione)(DMSO)] (PdDMSOTTA) and [$Pd^{2+}Cl_2$(4,4,4-trifluoro-1-phenyl-1,3-butanedione)(DMSO)] (PdDMSOBTA). The third chapter aimed to investigate the activity of platinum complexes bound to β-diketones, with the formulas [$Pt^{2+}Cl_2$(4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione)(DMSO)] (PtDMSOTTA) and [$Pt^{2+}Cl_2$(4,4,4-trifluoro-1-phenyl-1,3-butanedione)(DMSO)] (PtDMSOBTA). The tests were conducted on six *C. jejuni* strains resistant to erythromycin and ciprofloxacin, evaluated in both planktonic and sessile forms. The bactericidal activity of the complexes was determined through the minimum bactericidal concentration (MBC). All strains were classified according to the biofilm formation index (BFI) as non-producers, weak, moderate, or strong biofilm producers, while antibiofilm activity was evaluated through mature biofilm eradication assays. The ultrastructure of the biofilms was analyzed by scanning electron microscopy, the metabolomic profile by gas chromatography coupled with mass spectrometry (GC–MS), and cytotoxicity in differentiated Caco-2 cells by the resazurin reduction assay. The tests presented in Chapter Two show the results for palladium complexes coordinated to β-diketones. The PdDMSOBTA complex exhibited greater bactericidal activity against planktonic cells, with an average MBC of 15.36 µg/mL, eliminating all strains at concentrations < 50 µg/mL, whereas PdDMSOTTA showed lower efficacy. In biofilms, PdDMSOBTA promoted complete elimination at concentrations < 400 µg/mL and significantly reduced the adhered biomass, with an average reduction of approximately 75% in the biofilm formation index. Metabolomic analysis revealed alterations in metabolic pathways related to amino acid metabolism, purine metabolism, and cellular stress response. The viability of Caco-2 cells remained above 80–90% after exposure to the compounds, indicating low cytotoxicity. In Chapter Three, the antimicrobial potential of platinum(II) β-diketonate complexes, PtDMSOBTA and PtDMSOTTA, was investigated against the same six resistant strains of *C. jejuni*. The PtDMSOBTA complex showed higher bactericidal activity in the planktonic form, with an average MBC of 12.76 µg/mL, while PtDMSOTTA showed lower efficacy. In biofilms, PtDMSOBTA eliminated all isolates at concentrations < 200 µg/mL and significantly reduced biofilm biomass, whereas PtDMSOTTA showed a moderate effect. Scanning electron microscopy revealed structural disorganization of the biofilms and reduction of the extracellular matrix. Metabolomic analysis identified alterations in pathways related to amino acid metabolism, redox balance, and tryptophan metabolism. Both complexes showed low cytotoxicity, with cell viability between 80 and 100%. The study allowed the conclusion that palladium(II) and platinum(II) β-diketonate complexes, especially PdDMSOBTA and PtDMSOBTA, containing the ligand 4,4,4-trifluoro-1-phenyl-1,3-butanedione, exhibit significant antimicrobial activity against *C. jejuni* in both planktonic and sessile forms. For

planktonic cells, concentrations up to 100 µg/mL were sufficient to eliminate the microorganism, possibly due to metabolic alterations associated with the induction of cellular stress. In addition, the compounds showed low cytotoxicity in eukaryotic cells, indicating an initial profile of biological safety. Regarding the sessile form, higher concentrations were required for biofilm eradication, and additional cytotoxicity studies are still necessary to confirm safety under these conditions. Overall, the results obtained highlight the potential of these metal complexes as promising alternatives for the control of *C. jejuni*, especially in strategies aimed at reducing contamination in environments related to food production and processing.

Keywords: Bacterial Resistance; Biofilms; Cytotoxicity; Poultry Farming; Metabolomics.

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CAPÍTULO I
Considerações gerais

1. INTRODUÇÃO

A segurança microbiológica dos alimentos representa um dos principais desafios para a saúde pública global. Doenças transmitidas por alimentos (DTAs) continuam sendo responsáveis por elevada morbimortalidade em diferentes regiões do mundo. Estas doenças afetam milhões de pessoas anualmente e geram impactos significativos nos sistemas de saúde e na cadeia produtiva de alimentos. Estima-se que, apenas nos Estados Unidos da América (EUA), aproximadamente 48 milhões de pessoas adoeçam a cada ano devido em decorrência de infecções por DTAs, o que resulta em cerca de 128 mil hospitalizações e aproximadamente 3 mil mortes anuais, de acordo com estimativas do *Centers for Disease Control and Prevention* (CDC) (CDC, 2025a).

Na Europa, dados da *European Food Safety Authority* (EFSA), em conjunto com o *European Center for Disease Control* (ECDC), indicam que milhares de surtos alimentares são registrados anualmente nos países da União Europeia, com mais de 5.600 surtos relatados em 2023, resultando em mais de 52 mil casos humanos, cerca de 2.894 hospitalizações e 65 mortes associadas a doenças transmitidas por alimentos (ECDC, 2025).

Apesar dos avanços nos sistemas de vigilância e controle sanitário, as DTAs representam um risco para a saúde pública e para os sistemas de produção e processamento de alimentos (Atasever, 2026). Entre os agentes etiológicos associados a essas infecções, bactérias patogênicas de origem alimentar e suas toxinas são capazes de persistir em ambientes de processamento e na cadeia de produção animal (Bintsis, 2017). Em 2024, as zoonoses mais notificadas em humanos foram a campilobacteriose e a salmonelose, respetivamente, seguidas pelas infeções por *Escherichia coli* produtora de toxina Shiga (STEC) (ECDC, 2025).

No gênero *Campylobacter, C. jejuni* lidera em países da União Européia e EUA como o principal agente causador de gastroenterite em humanos (Kaakoush et al., 2015a). A infecção por essa bactéria está frequentemente associada ao consumo ou à manipulação de carne de frango contaminada, uma vez que aves domésticas constituem um dos principais reservatórios do microrganismo (Hermans et al., 2012). A colonização intestinal de aves ocorre geralmente de forma assintomática, favorecendo a disseminação do patógeno ao longo da cadeia produtiva e aumentando o risco de contaminação de carcaças durante o processamento (Bintsis, 2017).

Além da elevada prevalência em produtos avícolas, a persistência de *C. jejuni* no ambiente de produção está associada à sua capacidade de formar biofilmes em diferentes superfícies. A formação de biofilmes confere maior proteção às células bacterianas contra condições ambientais adversas, agentes sanitizantes e antimicrobianos, contribuindo para a

sobrevivência do microrganismo em equipamentos e instalações de processamento de alimentos (Bronowski et al., 2014; Marques et al., 2012; Teh et al., 2014)

Nos últimos anos, compostos metálicos têm sido investigados como alternativas no desenvolvimento de novos agentes antimicrobianos. Complexos metálicos apresentam propriedades químicas e estruturais que podem favorecer diferentes interações com biomoléculas celulares, incluindo proteínas, ácidos nucleicos e componentes da membrana bacteriana (Lemire et al., 2013a). Essas interações podem resultar em múltiplos mecanismos de ação, o que tem despertado interesse na utilização desses compostos como estratégia para contornar limitações associadas aos antimicrobianos convencionais. Nesse contexto, complexos de metais de transição, como platina e paládio têm demonstrado atividade antimicrobiana contra diferentes microrganismos, incluindo bactérias associadas a infecções humanas e patógenos de origem alimentar (Frei et al., 2020a). A atividade biológica desses complexos está frequentemente relacionada à natureza do metal central, à geometria do complexo e às características estruturais dos ligantes coordenados, fatores que influenciam diretamente sua estabilidade, reatividade e interação com alvos celulares (Russo et al., 2020).

2. OBJETIVOS

2.1. Objetivo Geral

Avaliar a atividade antimicrobiana de complexos metálicos de paládio(II) e platina(II) contendo ligantes β-dicetonas frente a *C. jejuni*, investigando seus efeitos sobre células planctônicas e biofilmes bacterianos, bem como as alterações metabólicas induzidas pela exposição bacteriana e a citotoxicidade em células eucarióticas.

2.2. Objetivos Específicos

i. Avaliar a atividade antimicrobiana desses complexos metálicos frente a *C. jejuni* em células planctônicas, determinando parâmetros de sensibilidade bacteriana;
ii. Investigar o efeito dos complexos de paládio(II) e platina(II) sobre o tratamento de biofilmes formados de *C. jejuni*;
iii. Avaliar as alterações metabólicas induzidas em *C. jejuni* após exposição aos complexos metálicos por meio de análises metabolômicas;
iv. Determinar a citotoxicidade dos complexos em células Caco-2 diferenciadas como modelo de epitélio intestinal, visando estimar o potencial de segurança biológica desses compostos.

3. REVISÃO BIBLIOGRÁFICA

3.1. Doenças Transmitidas por Alimentos

As doenças transmitidas por alimentos (DTAs) são definidas como enfermidades causadas pela ingestão de alimentos ou água contaminados por microrganismos patogênicos, toxinas ou substâncias químicas capazes de provocar alterações no estado de saúde do consumidor. Esses agravos representam um importante problema de saúde pública mundial, pois afeta populações em diferentes níveis de desenvolvimento socioeconômico e gera impactos significativos nos sistemas de saúde e na cadeia produtiva de alimentos (WHO, 2026).

Os agentes etiológicos associados às DTAs incluem bactérias, vírus, parasitas e toxinas microbianas, sendo as bactérias responsáveis por grande parte dos casos reportados globalmente. Entre os principais patógenos bacterianos associados às DTAs, destacam-se *Campylobacter spp.*, *Salmonella spp.*, *Escherichia coli* produtora de toxina Shiga, *Listeria monocytogenes* e *Staphylococcus aureus*, microrganismos frequentemente relacionados a surtos alimentares e a infecções esporádicas em humanos (ECDC, 2025; Scallan et al., 2011).

A contaminação dos alimentos pode ocorrer em diferentes etapas da cadeia produtiva, incluindo produção primária, processamento, armazenamento, transporte e preparo para consumo. Produtos de origem animal, como carnes, leite e ovos, estão entre as principais fontes de transmissão de patógenos alimentares, embora alimentos de origem vegetal também possam atuar como veículos importantes de infecção quando contaminados durante o cultivo ou processamento. Além disso, práticas inadequadas de higiene, manipulação e armazenamento dos alimentos podem favorecer a sobrevivência e multiplicação de microrganismos patogênicos (Havelaar et al., 2015).

3.2. Epidemiologia

A magnitude do problema das DTAs é evidenciada por dados epidemiológicos globais. Estimativas da Organização Mundial da Saúde indicam que, anualmente, cerca de 600 milhões de pessoas adoecem em decorrência do consumo de alimentos contaminados, resultando em aproximadamente 420 mil mortes em todo o mundo (Havelaar et al., 2015; WHO, 2026). Nos EUA, estima-se que aproximadamente 48 milhões de casos de DTAs ocorram a cada ano, resultando em cerca de 128 mil hospitalizações e 3 mil mortes (Scallan et al., 2011). Na União Europeia, relatórios recentes também apontam milhares de surtos alimentares anuais (ECDC,

2025). Na Inglaterra, por exemplo, o número de casos confirmados aumentou de 60.055 em 2023 para 70.352 em 2024, correspondendo a um acréscimo de 10.297 casos (17,1%). Esse aumento foi acompanhado por elevação na taxa de notificação, que passou de 104,1 para 121,9 casos por 100.000 habitantes (UK.GOV, 2026)

Em escala europeia, foram registrados 137.107 casos confirmados de campilobacteriose em 2022, resultando em uma taxa de notificação de 43,1 casos por 100.000 habitantes. Além dos dados epidemiológicos em humanos, informações provenientes de monitoramento de segurança alimentar indicam ampla presença de *Campylobacter* em diferentes cadeias produtivas. No critério de higiene de processo aplicado a alimentos segundo a legislação da União Europeia 2017/1495, em que amostragens são feitas de forma regular, aproximadamente 38–39% das amostras analisadas apresentaram resultado positivo para *Campylobacter* spp. A detecção foi relativamente baixa em alimentos prontos para consumo, porém significativamente maior em alimentos não prontos, particularmente em carnes e produtos cárneos e, nestes produtos, chama-se a atenção para a contaminação cruzada que pode ocorrer em domicílios. Entre essas categorias, as maiores taxas de contaminação foram observadas em carnes de frango e peru (UE, 2017; UK.GOV, 2026).

No Brasil, a vigilância das DTAs é realizada pelo Ministério da Saúde por meio do Sistema de Vigilância Epidemiológica das Doenças Transmitidas por Alimentos (VE-DTA), que monitora surtos e investiga os fatores envolvidos na ocorrência desses eventos. De acordo com dados oficiais, milhares de surtos de DTAs são registrados no país ao longo dos anos, envolvendo diferentes agentes etiológicos e alimentos contaminados. Entre os principais agentes identificados destacam-se bactérias como *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus* e *Bacillus cereus*, contrariando as estatísticas observadas a nível global e aponta o nível de subnotificação do país (Brasil, 2025).

Além das ações de vigilância epidemiológica, o controle das DTAs no país também é sustentado por legislações sanitárias que estabelecem padrões microbiológicos e boas práticas de fabricação para alimentos. Nesse contexto, destacam-se as normas publicadas pela Agência Nacional de Vigilância Sanitária (ANVISA), como a Resolução da Diretoria Colegiada RDC nº 331/2019, que define os padrões microbiológicos para alimentos, e a Instrução Normativa nº 60/2019, que estabelece listas de microrganismos e limites microbiológicos aceitáveis em diferentes categorias de alimentos. Essas regulamentações visam reduzir riscos microbiológicos ao consumidor e fortalecer as estratégias de segurança alimentar ao longo da cadeia produtiva (Brasil, 2025; Brasil, 2019; Brasil, 2019; Brasil, 2021).

Entre os patógenos bacterianos associados às DTAs, espécies do gênero *Campylobacter* se destacam. Em diversos países da Europa e da América do Norte, *C. jejuni* é considerado o principal agente responsável por infecções gastrointestinais associadas ao consumo de alimentos contaminados, especialmente produtos avícolas, o que ressalta a importância do estudo desse microrganismo no contexto da segurança microbiológica dos alimentos (FSIS, 2026; Kaakoush et al., 2015a; UK.GOV, 2026).

3.3. O Gênero *Campylobacter*: História e Taxonomia

O gênero *Campylobacter* compreende bactérias Gram-negativas pertencentes à família *Campylobacteraceae*, amplamente distribuídas na natureza e frequentemente associadas ao trato gastrointestinal de animais domésticos e silvestres. Esses microrganismos são reconhecidos como importantes agentes de doenças gastrointestinais em humanos e animais, e são considerados como principais patógenos bacterianos associados às DTAs em diversos países (Kaakoush et al., 2015a).

Historicamente, organismos semelhantes a *Campylobacter* sp. foram inicialmente observados no início do século XX em casos de aborto em ovinos e bovinos, e classificados na época como pertencentes ao gênero *Vibrio* devido à sua morfologia curva e motilidade característica. Apenas na década de 1960 esses microrganismos foram reclassificados em um novo gênero, denominado *Campylobacter*, após estudos que demonstraram diferenças fisiológicas e bioquímicas em relação às espécies de *Vibrio* (Vandamme et al., 1991). O avanço das técnicas de cultivo seletivo e de métodos de identificação microbiológica permitiu posteriormente a associação dessas bactérias com infecções gastrointestinais humanas. Desde então o gênero *Campylobacter* tem notável interesse em saúde pública (Silva et al., 2011).

As bactérias do gênero *Campylobacter* apresentam morfologia característica em forma de bastonetes curvos ou espiralados, podendo assumir formato semelhante a “asa de gaivota” quando observadas ao microscópio. São microrganismos móveis devido à presença de um ou dois flagelos polares, característica que contribui para sua capacidade de colonização e invasão do epitélio intestinal. Em termos fisiológicos, são bactérias microaerófilas, requerendo concentrações reduzidas de oxigênio (aproximadamente 5%) e níveis elevados de dióxido de carbono para crescimento ideal. Além disso, apresentam crescimento ótimo em temperaturas próximas a 42 °C, característica associada à adaptação ao trato intestinal de aves, que constituem um dos principais reservatórios naturais dessas bactérias (Humphrey et al., 2007; Silva et al., 2011).

Atualmente, o gênero *Campylobacter* inclui diversas espécies, das quais algumas apresentam maior relevância clínica e epidemiológica. Entre as espécies mais frequentemente associadas a infecções humanas destacam-se *C. jejuni*, *C. coli*, *C. lari* e *C. upsaliensis*. Dentre essas, *C. jejuni* é responsável pela maioria dos casos de gastroenterite bacteriana em humanos, seguido por *C. coli*, que também tem sido isolado em surtos e casos esporádicos de infecção alimentar. Essas espécies estão frequentemente associadas ao consumo de alimentos contaminados, especialmente produtos avícolas, leite cru e água contaminada (Atasever, 2026; Blankenship & Craven, 1982; Humphrey et al., 2007).

Entre as espécies do gênero, *C. jejuni* destaca-se pela elevada prevalência em infecções humanas e pela forte associação com a cadeia produtiva de aves. Em função dessa relevância epidemiológica, essa espécie tem sido amplamente estudada em relação aos seus mecanismos de virulência, estratégias de sobrevivência no ambiente e capacidade de persistência em sistemas de produção de alimentos (Takeuchi et al., 2022).

3.4. Legislação e Epidemiologia

Estudos epidemiológicos indicam que a maioria dos casos está relacionada à cadeia produtiva avícola, uma vez que aves domésticas atuam como importantes reservatórios naturais do microrganismo. A contaminação pode ocorrer durante o abate e processamento das aves, resultando na presença da bactéria em produtos cárneos destinados ao consumo humano (Takeuchi et al., 2022).

Em países europeus e na América do Norte, *C. jejuni* é responsável por um número significativo de casos de gastroenterite bacteriana, e é monitorada em sistemas de vigilância epidemiológica. A alta incidência está relacionada tanto à ampla distribuição do microrganismo em reservatórios animais quanto à sua capacidade de sobrevivência, à capacidade de formar biofilmes e à aquisição da forma viável, mas não cultivável (VNC) (Atasever, 2026; CDC, 2025; ECDC, 2025). Na forma VNC, *C. jejuni* representa um desafio crítico para a indústria avícola, pois permite que a bactéria sobreviva a condições ambientais adversas (estresse por oxigênio, baixas temperaturas e saneantes) durante o processamento de carcaças, mantendo sua patogenicidade, embora não cresça em meios de cultura convencionais (Atasever, 2026).

A vigilância epidemiológica no Brasil possui base legal estabelecida pela Lei nº 6.259, de 30 de outubro de 1975, que organiza as ações de vigilância epidemiológica no país e determina a notificação compulsória de doenças transmissíveis às autoridades de saúde pública. Essa legislação foi regulamentada pelo Decreto nº 78.231, de 1976, que definiu os mecanismos

operacionais para a notificação e o acompanhamento dessas enfermidades no território nacional (BRASIL, 1975).

Ao longo das últimas décadas, o Ministério da Saúde atualizou periodicamente a Lista Nacional de Notificação Compulsória com o objetivo de incorporar novas doenças e aprimorar os mecanismos de vigilância. A Portaria GM/MS n.º 5.201, de 15 de agosto de 2024, representa a atualização mais recente desse instrumento normativo e ampliou o escopo de doenças monitoradas no país. Entretanto, a campilobacteriose não figura de forma destacada nessa lista, o que limita a sistematização da vigilância epidemiológica e contribui para a subnotificação dessa infecção no Brasil (BRASIL, 2024).

Como consequência, o país apresenta escassez de dados consolidados sobre a epidemiologia da campilobacteriose. Estudos apontam que a ausência de um sistema de vigilância específico, associada à baixa notificação laboratorial, dificulta a estimativa real da incidência e da prevalência da doença no território nacional. Nesse contexto, a campilobacteriose permanece considerada uma enfermidade negligenciada no Brasil, com poucos registros oficiais sobre sua ocorrência (Bessa et al., 2020).

Embora os dados epidemiológicos sejam limitados, estudos conduzidos na cadeia produtiva de alimentos indicam ampla circulação do patógeno. Em investigação realizada em abatedouros do sul do Brasil, *Campylobacter* spp. foi isolado em 37,1% das carcaças de frango analisadas, com predominância de *Campylobacter jejuni*, responsável por aproximadamente 97,5% dos isolados. Esses resultados reforçam o papel da carne de frango como importante veículo de transmissão para humanos (Takeuchi et al., 2022).

O controle sanitário de produtos de origem animal no Brasil ocorre sob responsabilidade do Ministério da Agricultura e Pecuária (MAPA). No âmbito federal, o Sistema de Inspeção Federal (SIF) fiscaliza estabelecimentos que produzem alimentos destinados ao comércio interestadual ou à exportação e assegura a conformidade com as normas sanitárias nacionais e internacionais (Brasil, 2026a). No nível subnacional, os Serviços de Inspeção Municipal (SIM) fiscalizam produtos destinados ao comércio local, enquanto os Serviços de Inspeção Estadual (SIE) atuam em estabelecimentos que comercializam produtos no território estadual.

Esses serviços podem integrar o Sistema Brasileiro de Inspeção de Produtos de Origem Animal (SISBI-POA), desde que atendam aos padrões estabelecidos pelo SIF. O SISBI-POA permite a harmonização das ações de inspeção sanitária em todo o país e autoriza a comercialização nacional de produtos provenientes de estabelecimentos reconhecidos pelo sistema (Brasil, 2024; Brasil, 2025).

Apesar dessa estrutura normativa consolidada para a inspeção sanitária de alimentos, a legislação brasileira não estabelece critérios microbiológicos específicos ou limites quantitativos para *Campylobacter* em matrizes cárneas, como carcaças de aves, carnes bovinas ou suínas. Dessa forma, o controle desse patógeno ocorre de maneira indireta, por meio da adoção de boas práticas de fabricação, programas de Análise de Perigos e Pontos Críticos de Controle (HACCP) e monitoramento de indicadores gerais de higiene, além do atendimento a exigências sanitárias impostas por países importadores no contexto das exportações (Brasil, 2026b).

Mesmo na ausência de critérios microbiológicos específicos, evidências demonstram que *Campylobacter jejuni* representa risco relevante para a saúde pública brasileira. O patógeno já foi isolado em diferentes fontes alimentares e em diversas espécies animais, o que indica circulação ampla no ambiente e na cadeia produtiva de alimentos (Takeuchi et al., 2022).

Dados oficiais do Ministério da Saúde registraram 12 surtos de doenças transmitidas por alimentos associados a *Campylobacter* entre os anos de 2000 e 2023 no Brasil (Quetz et al., 2012). Entretanto, esse número provavelmente representa subestimativa da ocorrência real da doença, sobretudo em países que não possuem sistemas estruturados de vigilância epidemiológica para esse patógeno (do Nascimento Veras et al., 2018).

Esse cenário difere daquele observado em países de alta renda. Nos EUA e em diversos países da União Europeia, a campilobacteriose possui notificação compulsória e integra sistemas nacionais de vigilância epidemiológica. Além disso, esses países adotam metas e diretrizes específicas para a redução da prevalência do patógeno na cadeia produtiva de alimentos, especialmente em produtos avícolas(CDC, 2025b; ECDC, 2025; Liu et al., 2022).

Nos EUA, a incidência estimada alcançou aproximadamente 19,3 casos por 100.000 habitantes em 2023, o que posiciona *Campylobacter* entre as principais causas bacterianas de gastroenterite transmitida por alimentos em países desenvolvidos. Embora surtos representem apenas cerca de 1% das notificações, análises epidemiológicas demonstram que carnes não prontas para consumo correspondem a aproximadamente 79,7% dos veículos de transmissão identificados (CDC, 2025a).

Estudos internacionais também descrevem padrões sazonais na ocorrência da doença. Países como Canadá e Reino Unido registram aumento de casos durante o verão, período associado a práticas alimentares como churrascos e ao aumento da sobrevivência bacteriana em temperaturas mais elevadas (ECDC, 2025). No Canadá, a incidência manteve-se relativamente estável entre 2014 e 2019, com valores entre 25,4 e 28,6 casos por 100.000 habitantes e predominância de *C. jejuni*, responsável por 88% a 91% dos casos (Havelaar et al., 2015). Em

regiões sem sistemas oficiais de vigilância, como a Tailândia, estudos independentes identificaram *Campylobacter spp.* como o principal agente de diarreia do viajante, responsável por 43,8% dos casos registrados em militares (Liu et al., 2022).

No Brasil, os registros de casos clínicos humanos permanecem escassos. Em estudo conduzido no Nordeste do país, *C. jejuni* foi identificado em aproximadamente 16,4% das amostras fecais de crianças com diarreia, o que evidencia a importância do patógeno como agente etiológico nesse grupo populacional (Quetz et al., 2012). Investigações posteriores detectaram *C. jejuni* em cerca de 9,7% das amostras fecais avaliadas em estudos epidemiológicos com crianças e associaram a infecção a processos inflamatórios intestinais e possíveis impactos no estado nutricional infantil (do Nascimento Veras et al., 2018).

Dessa forma, *Campylobacter jejuni* destaca-se como um importante patógeno emergente associado às doenças transmitidas por alimentos, especialmente devido à sua ampla distribuição em reservatórios animais, à frequente contaminação de produtos avícolas e à crescente preocupação relacionada à resistência antimicrobiana.

3.5. Características Gerais, Virulência e Patogenia de *Campylobacter* Spp.

A infecção por *C. jejuni*, conhecida como campilobacteriose, é frequentemente associada ao consumo de alimentos contaminados, especialmente carne de frango mal cozida, leite não pasteurizado e água contaminada (El-Adawy & Hafez, 2024; WHO, 2020). A transmissão ocorre predominantemente pela via fecal-oral, podendo também estar relacionada ao contato direto com animais infectados ou com superfícies contaminadas durante o processamento de alimentos (El-Adawy & Hafez, 2024). Em humanos, a infecção geralmente se manifesta por diarreia, dor abdominal, febre, náuseas e, em alguns casos, pode evoluir para complicações pós-infecciosas, como a síndrome de Guillain-Barré (Gomes et al., 2021).

A Síndrome de Guillain-Barré (SGB) é uma das complicações pós-infecciosas mais relevantes associadas à infecção por *C. jejuni*. Trata-se de uma neuropatia inflamatória aguda do sistema nervoso periférico caracterizada por fraqueza muscular progressiva e paralisia flácida ascendente, que pode evoluir para comprometimento respiratório em casos graves (Latov, 2022). A patogênese da SGB associada a *C. jejuni* está relacionada principalmente ao mimetismo molecular, no qual lipooligossacarídeos presentes na superfície bacteriana apresentam estruturas semelhantes a gangliosídeos das células nervosas humanas. Essa semelhança estrutural pode induzir a produção de anticorpos que reagem de forma cruzada com componentes da mielina ou dos axônios periféricos, desencadeando uma resposta autoimune que resulta em dano neural. Estima-se que aproximadamente 20–40% dos casos de SGB

estejam associados a infecção prévia por *Campylobacter spp.* (Finsterer, 2022; Nayeem et al., 2025).

A patogenicidade de *C. jejuni* decorre da ação coordenada de fatores que permitem colonização intestinal, adesão e invasão epitelial, evasão imune e dano tecidual. Esses mecanismos incluem motilidade e quimiotaxia, adesão, invasão, produção de toxinas, secreção de proteínas efetoras e persistência bacteriana (Kemper & Hensel, 2023; Matilla & Krell, 2023). A interação entre esses fatores determina a capacidade do microrganismo de estabelecer infecção e desencadear os sinais clínicos característicos da campilobacteriose (Latov, 2022).

A motilidade constitui um dos primeiros determinantes envolvidos no processo infeccioso. A bactéria apresenta um ou dois flagelos polares que conferem elevada mobilidade em ambientes viscosos, como o muco intestinal. Essa característica facilita a travessia da camada de muco e permite o contato direto com as células epiteliais do intestino delgado e do cólon (Ramírez-Larrota & Eckhard, 2022).

Após alcançar o epitélio intestinal, *C. jejuni* utiliza proteínas de adesão específicas para se ligar às células do hospedeiro. Entre os principais fatores de adesão destacam-se as proteínas CadF, FlpA, JlpA e PebA, que se ligam a componentes da matriz extracelular, especialmente à fibronectina presente na superfície das células epiteliais intestinais (He et al., 2024).

A proteína CadF (*Campylobacter* adhesion to fibronectin) é considerada um dos principais determinantes de virulência, pois medeia a ligação direta da bactéria à fibronectina, promovendo uma interação inicial essencial para o processo de invasão celular (Bunduruș et al., 2023; He et al., 2024). A adesão eficiente às células epiteliais não apenas facilita a colonização, mas também desencadeia alterações na sinalização celular do hospedeiro, que favorecem a internalização bacteriana(Bunduruș et al., 2023; Hussein, 2018).

Após a adesão, *C. jejuni* é capaz de invadir células epiteliais intestinais. Esse processo envolve proteínas efetoras conhecidas como antígenos de invasão de *Campylobacter* (Cia), especialmente CiaB e CiaI, que são secretadas através de um sistema de secreção associado ao flagelo, funcionalmente semelhante ao sistema de secreção tipo III (Bunduruș et al., 2023).

Essas proteínas modulam a reorganização do citoesqueleto das células hospedeiras, permitindo a internalização da bactéria. Após a invasão, *C. jejuni* pode sobreviver em compartimentos intracelulares denominados vacúolos contendo *Campylobacter* (CCVs). Esses vacúolos apresentam características de endossomos precoces e tardios, mas evitam a fusão com lisossomos, permitindo a sobrevivência bacteriana dentro da célula (Tikhomirova et al., 2024). A capacidade de sobreviver intracelularmente contribui para a persistência da infecção e dificulta a eliminação do patógeno pelo sistema imune.

Entre os principais fatores de virulência destaca-se a toxina distensora citoletal (*Cytolethal Distending Toxin* – CDT), codificada pelos genes cdtA, cdtB e cdt*C.* Essa toxina constitui importante determinante de dano celular durante a infecção. O complexo holotóxico é composto por três subunidades: CdtA e CdtC participam do reconhecimento e da ligação às células-alvo, enquanto CdtB apresenta atividade semelhante à DNAse e induz quebras no DNA das células hospedeiras. dano genético provoca interrupção do ciclo celular, principalmente na fase G2/M, o que leva à distensão celular, apoptose ou morte celular programada. Além disso, a toxina estimula vias de sinalização inflamatórias, como NF-κB, que promovem a produção de citocinas pró-inflamatórias, incluindo IL-8, IL-6 e TNF-α (Gu et al., 2022). A inflamação resultante contribui para os sinais clínicos da campilobacteriose, como diarreia inflamatória e dor abdominal.

Estruturas superficiais bacterianas também exercem papel relevante na virulência. Entre essas estruturas destacam-se o lipooligossacarídeo (LOS), a cápsula polissacarídica e proteínas de membrana externa. Esses componentes participam da evasão da resposta imune e favorecem a sobrevivência bacteriana no hospedeiro (Bunduruș et al., 2023).

Essas proteínas modulam a reorganização do citoesqueleto das células hospedeiras, permitindo a internalização da bactéria. Após a invasão, *C. jejuni* pode sobreviver compartimentos intracelulares denominados vacúolos contendo *Campylobacter* (CCVs). Esses vacúolos apresentam características de endossomos precoces e tardios, mas evitam a fusão com lisossomos, permitindo a sobrevivência bacteriana dentro da célula (Tikhomirova et al., 2024). A capacidade de sobreviver intracelularmente contribui para a persistência da infecção e dificulta a eliminação do patógeno pelo sistema imune.

Esse dano genético resulta na interrupção do ciclo celular, principalmente na fase G2/M, levando à distensão celular, apoptose ou morte celular programada. Além disso, a toxina pode induzir respostas inflamatórias por meio da ativação de vias de sinalização celular, como NF-κB, resultando na produção de citocinas pró-inflamatórias, incluindo IL-8, IL-6 e TNF-α (Gu et al., 2022; Sales, 2022).

Estruturas superficiais bacterianas também desempenham papel importante na virulência de *C. jejuni*. Entre essas estruturas destacam-se o lipooligossacarídeo (LOS), a cápsula polissacarídica e proteínas de membrana externa. Essas estruturas estão associadas à evasão do sistema imunológico e à sobrevivência da bactéria no hospedeiro. O LOS apresenta estruturas moleculares que mimetizam gangliosídeos humanos, fenômeno conhecido como mimetismo molecular. A cápsula polissacarídica contribui para a resistência à fagocitose e à

ação do sistema complemento, favorecendo a persistência bacteriana durante a infecção (Latov, 2022).

A interação entre *C. jejuni* e o epitélio intestinal também desencadeia intensa resposta inflamatória local. A presença bacteriana ativa receptores do sistema imune inato presentes nas células epiteliais e em células do sistema imune da mucosa intestinal. Entre esses receptores destacam-se os receptores do tipo Toll (TLRs), que reconhecem componentes bacterianos como flagelina, lipooligossacarídeos e outras estruturas de superfície. A ativação dessas vias resulta na indução de cascatas de sinalização intracelular e na produção de mediadores inflamatórios(Hazel & Davies, 2022; Khan et al., 2023).

Entre os mediadores produzidos destacam-se citocinas pró-inflamatórias e quimiocinas, como interleucina-8 (IL-8), interleucina-6 (IL-6) e fator de necrose tumoral alfa (TNF-α). Essas moléculas promovem recrutamento de neutrófilos e outras células inflamatórias para o sítio de infecção. O acúmulo dessas células na mucosa intestinal contribui para o dano epitelial e para o desenvolvimento do quadro clínico característico da campilobacteriose, que inclui diarreia inflamatória, dor abdominal e febre (Bunduruș et al., 2023; Latov, 2022).

Em alguns casos, o processo inflamatório leva à formação de lesões na mucosa intestinal e à presença de sangue e leucócitos nas fezes. A intensidade dos sintomas depende de diversos fatores, incluindo a dose infectante, o estado imunológico do hospedeiro e as características genéticas da cepa envolvida. Indivíduos imunocomprometidos, crianças e idosos apresentam maior risco de desenvolver quadros mais graves ou prolongados (Acheson & Allos, 2001; Kaakoush et al., 2015b).

Além das manifestações gastrointestinais, infecções por *C. jejuni* podem desencadear complicações pós-infecciosas. Entre essas complicações destaca-se a síndrome de Guillain-Barré, uma neuropatia autoimune caracterizada por desmielinização progressiva dos nervos periféricos (Humphrey et al., 2007). A associação entre campilobacteriose e essa síndrome relaciona-se ao mimetismo molecular presente em determinadas estruturas de lipooligossacarídeos bacterianos, que apresentam similaridade estrutural com gangliosídeos humanos. A resposta imune gerada contra esses componentes bacterianos pode reagir de forma cruzada com tecidos do sistema nervoso periférico, o que resulta em dano neural (Finsterer, 2022).

A diversidade genética observada entre cepas de *C. jejuni* também exerce influência significativa sobre a virulência e a capacidade de adaptação do microrganismo. O genoma da espécie apresenta elevada plasticidade genética, com variação na presença e na expressão de genes associados à motilidade, adesão, invasão e produção de toxinas (Gu et al., 2022). Esse

repertório genético contribui para diferenças na capacidade de colonização, na intensidade da resposta inflamatória e na gravidade das manifestações clínicas observadas em diferentes infecções (Gu et al., 2022).

Além dos mecanismos clássicos de virulência, *C. jejuni* apresenta capacidade de formar biofilmes, característica que favorece sua sobrevivência em ambientes externos, superfícies alimentares e equipamentos utilizados no processamento de alimentos. Essa capacidade contribui para a persistência do patógeno ao longo da cadeia produtiva e aumenta o risco de transmissão ao ser humano (Melo et al., 2017; Püning et al., 2021).

Apesar de ser uma bactéria microaerófila e relativamente sensível a condições ambientais adversas, a organização em biofilmes aumenta sua tolerância a diferentes estresses físicos, químicos e nutricionais (Sung et al., 2026). Em ambientes industriais, essa estratégia favorece a permanência do microrganismo em superfícies frequentemente presentes em unidades de processamento de alimentos, como aço inoxidável, plástico e vidro(Teh et al., 2014). Nesse estado, as células bacterianas permanecem envoltas em uma matriz extracelular composta por polissacarídeos, proteínas, lipídios e DNA extracelular. Essa matriz forma uma estrutura organizada que oferece proteção contra dessecamento, agentes antimicrobianos e sanitizantes utilizados na indústria alimentícia (Ramírez-Larrota & Eckhard, 2022).

A formação de biofilme ocorre em etapas sucessivas. Inicialmente, as células bacterianas aderem a superfícies bióticas ou abióticas. Em seguida, ocorre agregação celular e produção de matriz extracelular, processo que culmina na maturação da estrutura tridimensional do biofilme. Na fase final, parte das células se desprende da matriz e coloniza novos ambientes, o que favorece a disseminação do microrganismo (Melo et al., 2017; Püning et al., 2021). Diversos fatores moleculares participam dessas etapas, incluindo estruturas relacionadas à motilidade e adesão, sistemas de resposta ao estresse e mecanismos de comunicação celular (Püning et al., 2021).

Entre os fatores associados à formação de biofilme destacam-se os flagelos, que desempenham papel central na motilidade e na adesão inicial às superfícies. Genes como *flaA* e *flaB*, responsáveis pela estrutura flagelar, são frequentemente relacionados à capacidade de formação de biofilmes. Além disso, proteínas de adesão como CadF, envolvida na ligação à fibronectina, também podem contribuir para a interação inicial com superfícies. Outros genes associados ao processo incluem *luxS*, relacionado ao sistema de quorum sensing; *sodB*, *katA* e *ahpC*, envolvidos na resposta ao estresse oxidativo; e genes relacionados à adaptação ao estresse térmico e ambiental, como *dnaJ* e *clpP* (Melo et al., 2017; Püning et al., 2021; Ramírez-Larrota & Eckhard, 2022).

As cepas de *C. jejuni* apresentam variações na capacidade de formar biofilmes, indicando que esse fenótipo é altamente cepa dependente. Pesquisas realizadas com isolados provenientes de abatedouros avícolas demonstraram que todas as cepas avaliadas foram capazes de formar biofilmes em superfícies como aço inoxidável e poliestireno, sob diferentes condições de temperatura e atmosfera (Teh et al., 2014). A formação foi observada tanto em condições microaerófilas quanto aeróbias, inclusive em temperaturas inferiores ao ótimo de crescimento (7 °C e 25 °C), o que sugere que o biofilme pode favorecer a sobrevivência da bactéria em ambientes industriais e de refrigeração (Melo et al., 2017).

A interação com outras bactérias também pode influenciar significativamente a formação de biofilmes por *C. jejuni*. A presença de bactérias como *Pseudomonas aeruginosa* ou *Escherichia coli* pode aumentar a densidade e a estabilidade estrutural do biofilme. Em biofilmes multiespécies, a densidade celular de *C. jejuni* frequentemente supera aquela observada em biofilmes formados por culturas puras, o que evidencia a importância das interações microbianas para a persistência desse patógeno em ambientes naturais e industriais (Jia et al., 2019; Lourenço et al., 2011).

Entre os fatores ambientais que influenciam esse processo, a temperatura exerce papel relevante. Experimentos que simulam condições de abatedouros demonstram níveis mais elevados de formação de biofilme em temperaturas próximas ao ótimo de crescimento da bactéria (42 °C). Entretanto, algumas cepas mantêm capacidade de formar biofilmes em temperaturas mais baixas, situação que representa risco adicional para a segurança alimentar em ambientes de processamento e armazenamento de produtos avícolas (Damtew et al., 2024).

O "*chicken juice*" consiste no exsudato liberado pela carne de frango durante o descongelamento ou processamento. Esse material contém proteínas, lipídios, carboidratos e outros componentes orgânicos provenientes dos tecidos musculares, que podem atuar como fonte de nutrientes e também como substrato facilitador da adesão bacteriana (Sung et al., 2026; Weerasooriya et al., 2022).

Microscopia eletrônica revelou que as células de *C. jejuni* frequentemente se aderem às partículas proteicas presentes no *chicken juice* depositadas sobre a superfície, em vez de aderirem diretamente ao material sólido. Dessa forma, o exsudato atua como uma camada condicionante ("*conditioning layer*"), modificando as propriedades físico-químicas da superfície e criando um ambiente mais favorável para a colonização bacteriana (Sung et al., 2024).

Outro aspecto relevante envolve a capacidade desse exsudato de compensar deficiências na motilidade bacteriana. Experimentos com mutantes aflagelados demonstraram que, mesmo

com motilidade reduzida, a presença de *chicken juice* restaurou parcialmente a formação de biofilme. Esse resultado sugere que o exsudato facilita a adesão bacteriana e contribui para a persistência do patógeno na cadeia de produção de alimentos (Sung et al., 2024; Weerasooriya et al., 2022).

Portanto, resíduos orgânicos provenientes da carne de frango presentes em equipamentos e superfícies industriais desempenham papel importante na persistência de *C. jejuni* em ambientes de processamento. A formação de biofilmes favorecida por esse material representa um desafio significativo para programas de higienização e controle microbiológico na indústria avícola, uma vez que essas estruturas podem aumentar a resistência da bactéria a sanitizantes e facilitar sua disseminação ao longo da cadeia alimentar (Blankenship & Craven, 1982; Peres et al., 2023; Sung et al., 2026).

3.6. Resistência Antimicrobiana em *Campylobacter jejuni*

Embora a maioria das infecções seja autolimitada, casos graves ou prolongados podem exigir terapia antimicrobiana, sendo os macrolídeos (principalmente eritromicina e azitromicina) considerados o tratamento de primeira linha, enquanto as fluoroquinolonas, como ciprofloxacina, já foram amplamente utilizadas como alternativa terapêutica (WHO, 2024). No entanto, o aumento da resistência a essas classes de antibióticos tem sido amplamente documentado em isolados clínicos, animais e ambientais, principalmente o uso desses antimicrobianos na medicina humana e na produção animal (Ahmed & Gulhan, 2022).

A resistência em *C. jejuni* ocorre por mutações cromossômicas que alteram os alvos bacterianos dos antibióticos, além da participação de sistemas de efluxo e, da aquisição de genes de resistência por transferência horizontal. Entre os antimicrobianos utilizados no tratamento da campilobacteriose, destacam-se a resistência aos macrolídeos e às fluoroquinolonas (Barata et al., 2024; Svensson et al., 2014).

Resistência às fluoroquinolonas

As fluoroquinolonas atuam inibindo enzimas essenciais para a replicação do DNA bacteriano, principalmente a DNA girase e a topoisomerase IV. Em *C. jejuni*, o principal mecanismo de resistência à ciprofloxacina está associado a mutações no gene *gyrA*, que codifica a subunidade A da DNA girase. Essas mutações ocorrem na região conhecida como QRDR

(quinolone resistance-determining region) e alteram o sítio de ligação do antibiótico, reduzindo sua eficácia (Payot et al., 2006; Shariati et al., 2022).

A mutação mais frequentemente descrita é a substituição Thr-86-Ile (T86I) na proteína GyrA, que confere níveis elevados de resistência à ciprofloxacina e ao ácido nalidíxico. Outras mutações também foram descritas, como Asp-90-Asn, Ala-70-Thr e Pro-104-Ser, podendo ocorrer isoladamente ou em combinação (Hooper & Jacoby, 2016). Em alguns casos, mutações múltiplas no gene *gyrA* ou em genes relacionados à topoisomerase IV podem aumentar ainda mais os níveis de resistência (Grossman et al., 2023).

Além das mutações no gene *gyrA*, a resistência às fluoroquinolonas pode ser intensificada pela ação de bombas de efluxo, especialmente o sistema CmeABC, que expulsa antibióticos do interior da célula bacteriana e reduz a concentração intracelular do fármaco. Esse sistema também está associado à resistência a múltiplos antimicrobianos e pode atuar de forma sinérgica com mutações cromossômicas para aumentar os níveis de resistência (Tsiklauri et al., 2022).

Em média, sistemas de monitoramento na União Europeia estimam prevalência de resistência próxima a 59% em isolados humanos de *C. jejuni*, embora existam variações regionais importantes. Em alguns países europeus, como a Itália, estudos clínicos recentes relatam níveis superiores a 70% de resistência à ciprofloxacina em isolados humanos. Dados de vigilância também indicam que cepas associadas a casos relacionados a viagens podem apresentar prevalências ainda mais elevadas, chegando a 85% de resistência em determinados contextos epidemiológicos (Veltcheva et al., 2022).

Em escala global, a resistência às fluoroquinolonas em *Campylobacter* tem aumentado nas últimas décadas, fenômeno amplamente atribuído ao uso extensivo dessa classe de antimicrobianos tanto na medicina humana quanto na produção animal, especialmente na avicultura. Em consequência desse cenário, a Organização Mundial da Saúde (OMS) e a European Food Safety Authority (EFSA) classificam a resistência às fluoroquinolonas em *Campylobacter* como uma crise de saúde pública global (EFSA, 2023; WHO, 2025).

Resistência aos macrolídeos (eritromicina)

Os macrolídeos constituem a principal classe de antimicrobianos utilizada no tratamento da campilobacteriose, especialmente em casos moderados a graves ou em pacientes pertencentes a grupos de risco. Fármacos como Eritromicina e Azitromicina atuam por meio da

ligação ao domínio V do RNA ribossomal 23S da subunidade 50S do ribossomo bacteriano. Essa interação bloqueia a translocação do ribossomo ao longo do RNA mensageiro e interrompe a síntese proteica, o que inibe o crescimento bacteriano (Bolinger & Kathariou, 2017a).

Em *Campylobacter jejuni*, o principal mecanismo de resistência aos macrolídeos envolve mutações pontuais no gene do 23S rRNA, especialmente nas posições 2074 e 2075. Essas alterações estruturais modificam o sítio de ligação do antibiótico no ribossomo e reduzem sua afinidade pelo alvo molecular, o que compromete sua atividade antimicrobiana. Entre essas mutações, a substituição A2075G representa o mecanismo mais frequentemente associado à resistência de alto nível em *C. jejuni* (Choi et al., 2021). Em muitos casos, essa mutação ocorre simultaneamente em todas as cópias do gene 23S rRNA presentes no genoma bacteriano, condição que resulta em níveis elevados de resistência e frequentemente se associa a valores de concentração inibitória mínima (MIC) superiores a 128 μg/mL (Bolinger & Kathariou, 2017a; Veltcheva et al., 2022).

Além das mutações no RNA ribossomal, outros mecanismos podem contribuir para o desenvolvimento ou intensificação do fenótipo resistente. Entre eles destacam-se os sistemas de efluxo, particularmente a bomba CmeABC, que reduz a concentração intracelular do antimicrobiano por meio da extrusão ativa da molécula para o meio extracelular. Alterações estruturais nas proteínas ribossomais L4 e L22 também podem interferir no sítio de ligação dos macrolídeos e diminuir sua eficácia (Liao et al., 2022). Outro mecanismo relevante envolve genes de metilação ribossomal, como erm(B), que promovem modificações químicas no RNA ribossomal e reduzem a afinidade do antibiótico pelo ribossomo bacteriano. A presença desses determinantes genéticos pode resultar em níveis variados de resistência e, em alguns casos, contribuir para a disseminação do fenótipo resistente entre diferentes linhagens bacterianas (Gao et al., 2023).

Apesar da existência desses mecanismos, a resistência aos macrolídeos em *C. jejuni* apresenta, de modo geral, prevalência inferior à observada para fluoroquinolonas. Estudos epidemiológicos indicam que as taxas de resistência à eritromicina em isolados clínicos humanos costumam variar entre aproximadamente 2% e 6% em muitas regiões do mundo (Engberg et al., 2001). Entretanto, valores mais elevados podem ocorrer em isolados de origem animal ou em áreas geográficas com uso mais intensivo de antimicrobianos na produção pecuária (Bolinger & Kathariou, 2017b).

Dados de vigilância internacional indicam que a resistência global aos macrolídeos permanece relativamente baixa quando comparada a outras classes de antimicrobianos. Em países da Europa e da América do Norte, a maioria dos sistemas de monitoramento reporta

prevalências inferiores a 10% em isolados humanos de *C. jejuni* (Engberg et al., 2001). No entanto, regiões da Ásia apresentam valores mais elevados, em alguns casos superiores a 15%, especialmente em isolados provenientes de produção avícola. Relatórios de organizações internacionais como a OMS e a EFSA indicam que, embora os macrolídeos permaneçam eficazes para o tratamento da campilobacteriose, o aumento gradual de cepas resistentes representa uma preocupação emergente para a saúde pública global (EFSA, 2023; WHO, 2025, 2026).

Tendências epidemiológicas da resistência

A resistência antimicrobiana é uma ameaça à saúde pública global. Estimativas indicam que aproximadamente uma em cada seis infecções bacterianas comuns apresenta algum nível de resistência aos tratamentos disponíveis, com aumento anual estimado entre 5% e 15% no período entre 2018 e 2023. Esse avanço progressivo de bactérias resistentes, frequentemente denominadas “superbactérias”, sobrecarrega os sistemas de saúde e pode resultar aumenta a morbidade e mortalidade associadas a infecções bacterianas (WHO, 2024). Estima-se que a resistência antimicrobiana tenha contribuído para cerca de 1,27 milhão de mortes em 2019, e projeções epidemiológicas sugerem que esse número poderá atingir até 10 milhões de óbitos anuais até 2050, caso medidas efetivas de controle não sejam implementadas (Liao et al., 2022).

Embora a resistência antimicrobiana seja um fenômeno global, sua carga é particularmente elevada em países de baixa e média renda, onde o acesso limitado a diagnósticos microbiológicos, a vigilância epidemiológica insuficiente e as condições sanitárias inadequadas favorecem a disseminação de microrganismos resistentes (Rony et al., 2023). Entre os principais fatores que impulsionam esse processo destacam-se o uso excessivo e inadequado de antibióticos na medicina humana e na produção animal, além de falhas em práticas de saneamento, higiene e controle de infecções. Do ponto de vista clínico e econômico, infecções causadas por bactérias resistentes estão associadas a hospitalizações prolongadas, aumento significativo dos custos em saúde e maiores taxas de mortalidade (Barata et al., 2024).

Diante da crescente limitação das opções terapêuticas convencionais, diferentes linhas de pesquisa têm investigado alternativas capazes de contornar os mecanismos clássicos de resistência bacteriana. Entre essas estratégias está o uso de compostos naturais, peptídeos antimicrobianos, nanopartículas e complexos metálicos com atividade antimicrobiana (Annamalai et al., 2013; Balta et al., 2021; Frei et al., 2020a). Esses compostos frequentemente apresentam múltiplos mecanismos de ação, incluindo geração de espécies reativas de oxigênio,

interação com biomoléculas celulares e interferência em processos metabólicos essenciais, o que pode reduzir a probabilidade de desenvolvimento de resistência (Frei et al., 2020; Lemire et al., 2013). Nesse contexto, os complexos metálicos têm despertado interesse crescente como potenciais agentes antimicrobianos, especialmente contra bactérias multirresistentes, abrindo novas perspectivas para o desenvolvimento de terapias alternativas no controle de infecções bacterianas.

3.7. Estratégias Alternativas ao Uso De Antibióticos: Uso de Metais como Agentes Antimicrobianos

O aumento global da resistência antimicrobiana tem impulsionado a busca por estratégias alternativas aos antibióticos convencionais. Entre essas abordagens, o uso de metais e complexos metálicos com atividade antimicrobiana tem recebido crescente atenção, devido ao seu amplo espectro de ação e aos múltiplos mecanismos pelos quais podem inibir ou eliminar microrganismos patogênicos (Do Couto Almeida et al., 2014; Lacerda et al., 2022a). Diferentemente dos antibióticos tradicionais, que geralmente possuem um único alvo molecular específico, os metais podem atuar simultaneamente sobre diferentes estruturas celulares bacterianas, dificultando o desenvolvimento de resistência.

Histórico do uso de metais como antimicrobianos

O uso de metais com propriedades antimicrobianas remonta à antiguidade. Registros históricos indicam que civilizações antigas, como os egípcios, gregos e romanos, utilizavam recipientes de prata e cobre para armazenar água e alimentos, observando empiricamente que esses materiais ajudavam a prevenir deterioração e doenças (Erkey & Türk, 2021). Hipócrates descreveu o uso de compostos de prata no tratamento de feridas, enquanto civilizações persas e fenícias utilizavam recipientes metálicos para prolongar a conservação da água (Alexander, 2009).

Durante o século XIX e início do século XX, compostos de metais passaram a ser utilizados de forma mais sistemática na medicina. Sais de prata foram amplamente empregados como antissépticos e no tratamento de infecções oculares em recém-nascidos, enquanto compostos de arsênio, como o Salvarsan, foram utilizados no tratamento da sífilis (Bosch & Rosich, 2008; Lansdown, 2006; Rai et al., 2009; Turner, 2017). Posteriormente, o desenvolvimento de antibióticos levou a uma redução no uso terapêutico de metais; entretanto,

nas últimas décadas, o interesse por esses compostos tem ressurgido devido ao avanço da resistência antimicrobiana (Turner, 2017).

Propriedades antimicrobianas dos metais

Diversos metais apresentam propriedades antimicrobianas naturais, incluindo prata, cobre, zinco, ouro, paládio e platina. A atividade antimicrobiana desses elementos está associada principalmente à sua capacidade de interagir com biomoléculas celulares essenciais (Turner, 2017).

Entre os principais mecanismos de ação propostos destacam-se: a interação com proteínas e enzimas bacterianas, levando à desnaturação ou inativação de funções metabólicas essenciais; Danos à membrana celular, resultando em aumento da permeabilidade e perda de integridade estrutural; Interação com ácidos nucleicos, causando interferência na replicação e transcrição do DNA; Geração de espécies reativas de oxigênio (ROS), que promovem estresse oxidativo e danos celulares (Lemire et al., 2013b).

A presença de múltiplos alvos celulares faz com que os metais apresentem atividade antimicrobiana de amplo espectro, atuando contra bactérias Gram-positivas, Gram-negativas, fungos e alguns vírus. Além disso, quando incorporados em complexos de coordenação, esses metais podem apresentar propriedades farmacológicas aprimoradas, como maior estabilidade química, maior seletividade biológica e aumento da atividade antimicrobiana (Afessa et al., 2010; Kollef et al., 2008; Nagar et al., 2023).

Complexos metálicos contendo metais de transição têm sido amplamente investigados como potenciais agentes antimicrobianos (Nies, 1999). Compostos contendo paládio e platina, por exemplo, apresentam estruturas químicas semelhantes às de compostos utilizados na quimioterapia anticâncer, como a cisplatina, e demonstram capacidade de interagir com biomoléculas bacterianas, incluindo DNA e proteínas. Essa interação pode levar à inibição da replicação celular e ao comprometimento de processos metabólicos essenciais (Do Couto Almeida et al., 2014b).

Aplicações na área de alimentos e controle microbiológico

Além das aplicações médicas, metais com propriedades antimicrobianas têm sido explorados na área de segurança alimentar e controle microbiológico. Superfícies contendo cobre ou prata, são utilizadas em ambientes hospitalares e em instalações de processamento de alimentos para reduzir a carga microbiana e minimizar a disseminação de patógenos.

Nanopartículas metálicas também têm sido investigadas para incorporação em embalagens antimicrobianas, com o objetivo de prolongar a vida útil de alimentos e reduzir o risco de contaminação (Grass et al., 2011; Vincent et al., 2016).

Aspectos regulatórios e legislação

Apesar do potencial antimicrobiano dos metais, seu uso é regulado por órgãos de saúde e segurança alimentar devido à possibilidade de toxicidade e acúmulo em organismos vivos. Órgãos de saúde internacionais estabeleceram limites para a presença de metais em alimentos, água e produtos farmacêuticos (Michels et al., 2009). A OMS e a EFSA estabelecem valores de ingestão diária tolerável para diversos metais, com base em avaliações toxicológicas. Nos EUA, a *Food and Drug Administration* (FDA) regula o uso de metais e compostos metálicos em medicamentos, dispositivos médicos e materiais em contato com alimentos (FDA, 2026; Tchounwou et al., 2012; WHO, 2007).

No Brasil, a regulamentação relacionada à presença de metais em alimentos e materiais de contato é estabelecida por órgãos como a Agência Nacional de Vigilância Sanitária (ANVISA) e o Ministério da Agricultura e Pecuária (MAPA). Essas instituições definem limites máximos de contaminantes metálicos em alimentos, além de regulamentar o uso de substâncias com atividade antimicrobiana em embalagens e superfícies de contato alimentar (BRASIL, 2021; BRASIL, 2019; Tchounwou et al., 2012).

Dessa forma, embora os metais apresentem grande potencial como agentes antimicrobianos alternativos, sua aplicação deve considerar cuidadosamente aspectos relacionados à segurança, toxicidade e regulamentação, garantindo que seu uso seja eficaz e seguro para a saúde humana e para o meio ambiente (WHO, 2007).

3.8. Atividade Bactericida do Paládio

Metais nobres têm sido amplamente investigados como alternativas aos antimicrobianos convencionais, e entre eles o paládio (Pd) tem despertado interesse crescente devido às suas propriedades catalíticas, estabilidade química e capacidade de interagir com biomoléculas celulares. Compostos contendo paládio, nanopartículas de paládio (PdNPs) e complexos metálicos, apresentam atividade bactericida contra *E. coli, S. aureus, Salmonella* spp. e *C. jejuni* (Bankier et al., 2019; Yang et al., 2021).

Sua ação já foi demonstrada por PdNPs sintetizadas por ablação a laser em meio líquido, onde observou-se atividade antibacteriana significativa contra *E. coli* e *S. aureus (Shabatina et al., 2022; Sylvestre et al., 2004)*; PdNPs por síntese biogênica contra diferentes microrganismos patogênicos, incluindo *Pseudomonas aeruginosa, Salmonella typhi, Bacillus cereus* e *E. coli* (Adams et al., 2014; Bankar et al., 2010); e PdNPs produzidas por biossíntese a partir de microalgas foram testadas contra bactérias multirresistentes, como *Staphylococcus aureus, Acinetobacter* spp. e *Pseudomonas aeruginosa*, apresentando concentrações inibitórias mínimas entre 62,5 e 125 μg/mL, o que demonstra potencial para aplicação no controle de patógenos resistentes (Dahoumane et al., 2016).

O mecanismo de dano celular do paládio está frequentemente associado à geração de espécies reativas de oxigênio (ROS), capazes de causar danos oxidativos a lipídios, proteínas e DNA bacteriano (Lemire et al., 2013c). O mecanismo de ação é mediado por estruturas cristalinas de paládio podem apresentar atividade enzimática semelhante à de oxidases e peroxidases, o que contribui para a geração dessas espécies reativas e consequente morte bacteriana. Além disso, a interação direta entre as nanopartículas e a membrana bacteriana pode resultar em aumento da permeabilidade celular, desestabilização da membrana e perda do potencial de membrana, levando à morte celular (Álvarez-Martínez et al., 2021).

A formação de biofilmes bacterianos representa um grande desafio para o controle microbiológico. Nesse contexto, a inibição da formação de biofilmes já foi observada após tratamento com PdNPs revestidas com biomoléculas naturais, com valores de IC_{50} próximos de 15 μg/mL para cepas de *Staphylococcus aureus*. Tentando solucionar esta problemática, o uso de superfícies funcionalizadas com nanopartículas de paládio demonstrou redução significativa na adesão bacteriana e na produção de polissacarídeos e proteínas que compõem a matriz extracelular do biofilme, indicando que o paládio pode interferir tanto na formação quanto na estabilidade estrutural dessas comunidades microbianas (Dahoumane et al., 2016; Fahmy et al., 2020).

Estudos comparativos avaliando diferentes metais com potencial antimicrobiano demonstraram que paládio, platina e ouro apresentam algumas das maiores atividades bactericidas entre os metais testados. Trabalhos com *Enterococcus faecium, Klebsiella pneumoniae* e *Acinetobacter baumannii*, soluções contendo íons metálicos mostraram forte atividade antimicrobiana tanto em células planctônicas quanto em biofilmes bacterianos (Harrison et al., 2004). As combinações de metais podem apresentar efeitos sinérgicos, aumentando ainda mais a eficácia antimicrobiana. Combinações como ouro/paládio ou

platina/paládio apresentaram maior capacidade de inibição bacteriana e de redução de biofilmes quando comparadas ao uso individual dos metais (Teng et al., 2024).

A possibilidade de modificar quimicamente o paládio por meio de complexos de coordenação ou nanopartículas funcionalizadas permite otimizar sua atividade biológica, estabilidade e seletividade (Do & Huynh, 2024). Por esse motivo, o desenvolvimento de novos compostos baseados em paládio tem sido amplamente explorado como estratégia promissora para o desenvolvimento de novos agentes antimicrobianos e antibiofilme, especialmente frente ao aumento da resistência bacteriana aos antibióticos convencionais (Adams et al., 2014; Do Couto Almeida et al., 2014b; Do & Huynh, 2024; Fahmy et al., 2020).

3.9. Platina: Caracterização Química e Estudos Comparativos de Atividade Antimicrobiana

A platina (Pt) é um metal de transição pertencente ao grupo 10 da tabela periódica, amplamente reconhecido por suas propriedades catalíticas, estabilidade química e capacidade de formar complexos de coordenação estáveis com diferentes ligantes. Essas características tornam a platina um elemento de grande interesse em diversas áreas da química, incluindo catálise, química medicinal e desenvolvimento de compostos com atividade biológica (Adams et al., 2014; Do & Huynh, 2024).

A platina apresenta múltiplos estados de oxidação, sendo Pt(II) e Pt(IV) os mais comuns em sistemas biológicos e compostos de coordenação. Entre esses, os complexos de Pt(II) são particularmente relevantes, pois apresentam geometria quadrado-planar, característica que favorece interações específicas com biomoléculas como proteínas, enzimas e ácidos nucleicos (Do & Huynh, 2024; Fahmy et al., 2020).

Os complexos de platina possuem alta afinidade por ligantes contendo átomos doadores de elétrons, como nitrogênio, enxofre e oxigênio, permitindo a formação de estruturas químicas estáveis com diferentes tipos de ligantes orgânicos e inorgânicos. Essa capacidade de coordenação contribui para a diversidade estrutural dos complexos de platina e influencia diretamente suas propriedades químicas e biológicas (Duan & Henkelman, 2021; Scalambra et al., 2021).

A importância dos complexos de platina na medicina tornou-se amplamente reconhecida após a descoberta da atividade antitumoral da cisplatina, um complexo de Pt(II) capaz de interagir com o DNA celular, causando danos estruturais que impedem a replicação e

levam à morte celular. A partir dessa descoberta, diversos outros complexos de platina foram desenvolvidos, principalmente para aplicações terapêuticas (Castañeda-Valencia et al., 2025).

Embora os compostos de platina sejam tradicionalmente associados à quimioterapia anticâncer, sua atividade antimicrobiana já foi previamente investigada (Johnstone et al., 2016). O mecanismo de ação desses compostos está frequentemente a interação com o DNA bacteriano, levando à formação de ligações cruzadas que impedem a replicação e transcrição; inibição de enzimas essenciais, especialmente aquelas que possuem resíduos de enxofre ou nitrogênio em seus sítios ativos; alterações na permeabilidade da membrana celular, comprometendo a integridade da célula bacteriana; indução de estresse oxidativo, resultante da geração de ROS (Blaskovich et al., 2016; Frei et al., 2020b).

Esses múltiplos mecanismos tornam os complexos de platina particularmente interessantes no contexto da resistência antimicrobiana, uma vez que a presença de diversos alvos celulares reduz a probabilidade de desenvolvimento de resistência bacteriana (Rosenberg et al., 1965). Quando usados em associação, a atividade antimicrobiana de complexos de platina com outros metais de transição, como paládio, ouro e prata, é potencializada. Em muitos casos, os complexos de platina apresentam atividade antimicrobiana comparável ou superior à de outros metais, especialmente quando associados a ligantes orgânicos capazes de aumentar a permeabilidade celular ou facilitar a interação com biomoléculas bacterianas (Teh et al., 2021).

4. CONSIDERAÇÕES FINAIS

Os complexos metálicos de platina e paládio apresentam propriedades químicas distintas que influenciam diretamente a atividade biológica desses metais. O paládio apresenta maior labilidade química, característica que favorece maior reatividade e, consequentemente, respostas biológicas mais rápidas. Em contraste, os complexos de platina apresentam maior estabilidade cinética, o que tende a promover interações mais controladas e prolongadas com biomoléculas. Como resultado, complexos de paládio frequentemente demonstram atividade antimicrobiana mais rápida, enquanto os de platina podem apresentar efeitos mais duradouros devido à maior estabilidade estrutural. Já a possibilidade de modificação estrutural da platina por meio da introdução de diferentes ligantes permite modular características como lipofilicidade, estabilidade e seletividade biológica. Nesse contexto, o desenvolvimento de novos complexos a base de paládio e platina tem sido amplamente investigado como estratégia para a identificação de agentes capazes de atuar contra patógenos resistentes e biofilmes bacterianos, ampliando as perspectivas terapêuticas diante do crescente desafio da resistência antimicrobiana.

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CAPITULO II

ANTIMICROBIAL EFFECT OF PALLADIUM(II) B-DIKETONATE COMPLEXES ON *Campylobacter jejuni* IN PLANKTONIC AND SESSILE FORMS

Antimicrobial effect of palladium(II) β-diketonate complexes on *Campylobacter jejuni* in planktonic and sessile forms

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Abstract

Campylobacter jejuni is a leading cause of foodborne gastroenteritis worldwide. Its persistence throughout the poultry production chain, combined with increasing antimicrobial resistance and biofilm formation, highlights the need for alternative control strategies. This study evaluated the antimicrobial activity of the palladium(II) β-diketonate complexes PdDMSOBTA and PdDMSOTTA against six resistant *C. jejuni* strains in planktonic and sessile forms, investigated metabolic alterations induced by the most active compound, and assessed its cytotoxicity. Five *C. jejuni* strains isolated from chicken carcasses in Brazil and one human reference strain (IAL) were included. Antimicrobial activity was determined by minimum bactericidal concentration (MBC), while antibiofilm activity was evaluated using the biofilm formation index (BFI), biofilm MBC, and scanning electron microscopy. GC–MS performed metabolomic profiling, and cytotoxicity was assessed in differentiated Caco-2 cells using the resazurin assay. PdDMSOBTA showed greater bactericidal activity against planktonic cells (mean MBC: 15.36 ± 17.31 µg/mL) than PdDMSOTTA (mean MBC: 37.81 ± 35.02 µg/mL), eliminating all strains at concentrations ≤50 µg/mL. Against biofilms, PdDMSOBTA achieved complete eradication at concentrations ≤400 µg/mL, reduced adhered biomass by approximately 75%, and reclassified strong biofilm producers as weak or non-producers. Metabolomic analysis revealed alterations in lipid, amino acid, purine, and detoxification pathways, together with increased stress-response metabolites. Cell viability remained above 80% after 24 and 48 h of exposure, indicating low cytotoxicity. Overall, palladium(II) β-diketonate complexes, particularly PdDMSOBTA, demonstrate strong antimicrobial activity against resistant *C. jejuni*, highlighting their potential as alternative antimicrobial agents in food production environments.

Keywords

Antimicrobial resistance; biofilm formation; electron microscopy; metabolomics; poultry industry

1 INTRODUCTION

Campylobacter spp. are enteric pathogens and are among the microorganisms most frequently involved in foodborne diseases worldwide (Ma et al., 2022). The poultry production chain is considered the link most strongly associated with food production and the epidemiology of foodborne campylobacteriosis (Tegtmeyer et al., 2021). In this context, the species that most commonly affect humans are *Campylobacter jejuni* and *Campylobacter coli* (Ansarifar et al., 2023).

This enteropathogen typically causes self-limiting diarrhea; however, infections may progress to severe disease in at-risk populations, such as pregnant women, older adults, and children (Buiatte et al., 2023). In these cases, campylobacteriosis may lead to serious outcomes, including sepsis, miscarriage, abscesses, and chronic sequelae such as Guillain–Barré syndrome (Mohan et al., 2025). Antimicrobial therapy is used to treat severe cases of campylobacteriosis, but its effectiveness has been increasingly compromised by the emergence of antimicrobial resistance and reduced susceptibility to commonly used compounds (Buiatte et al., 2024).

Antimicrobial resistance represents a significant public health concern and drives the search for new substances capable of controlling this pathogen (Dai et al., 2020). In poultry slaughterhouses, chicken meat, processing equipment, and wastewater generated during handling create favorable conditions for the persistence of *Campylobacter* spp. on surfaces (Rasschaert et al., 2020). In industrial settings, sanitizers are routinely applied for environmental control and disinfection in order to reduce microbiological contamination of the final product (Fernandes et al., 2025).

To control environmental contamination, several cleaning strategies are employed in food-processing facilities, including the use of easy-to-clean equipment, different concentrations of chemical agents, and temperature control (Dula et al., 2021). However, *Campylobacter* spp. possess mechanisms to cope with environmental stress, among which biofilm formation plays

a key role in resistance and persistence on surfaces (Gomes et al., 2024). Biofilms consist of a matrix of polymeric substances produced by one or more microbial species, promoting microbial growth, survival, and adhesion to surfaces (Tikhomirova et al., 2024).

Surfaces covered with biofilms can contaminate handled products, representing a significant source of infection for consumers (Hakeem & Lu, 2021). Moreover, the biofilm matrix facilitates microbial communication and horizontal gene transfer, contributing to the development and dissemination of antimicrobial resistance (Kim et al., 2021). Despite the widespread use of sanitizers and their recognized antimicrobial activity, these agents often show limited effectiveness in preventing biofilm formation, persistence, and regrowth in industrial environments (Rasschaert et al., 2020).

Given these limitations, new technologies for environmental disinfection have been investigated, with metal-based compounds emerging as promising alternatives for pathogen control in the food industry (Mohammed, 2025).

Palladium-based compounds have demonstrated inhibitory effects against bacteria associated with foodborne diseases and their biofilms (Chlumsky et al., 2021). Palladium may occur in metallic form or as Pd^{2+} and Pd^{4+} ions capable of coordination. Complexes formed by the coordination of Pd^{2+} ions with β-diketones and antibiotics have been widely reported to exhibit antitumor, antifungal, antimicrobial, and antiviral activities (Garoufis et al., 2009; Guerra et al., 2005). Although the activity of metal complexes against several foodborne pathogens has been described, the effects of palladium-containing compounds on *Campylobacter* spp., in both planktonic and sessile forms, remain poorly understood.

Therefore, this study aimed to simulate an industrial environment in vitro to evaluate the antimicrobial potential of palladium-based metal compounds against resistant strains of *C. jejuni*, assessing their effects on both planktonic cells and established biofilms. Additionally, we sought to determine inhibitory concentrations, evaluate the impact of these compounds on

biofilm formation and bacterial metabolism, and compare their efficacy with that of traditionally used sanitizers.

2 MATERIALS AND METHODS

2.1 Characterization of Palladium(II) β-Diketonate Metal Complexes

All metal complexes used in this study were synthesized and kindly provided by the Instituto de Química, Universidade Federal de Uberlândia (UFU). The compounds are palladium derivatives coordinated with β-diketone ligands, namely PdDMSOTTA (molar mass: 441.25 g/mol) and PdDMSOBTA (molar mass: 435.16 g/mol). These complexes have the general formula [MCl(L)DMSO], where L corresponds to 4,4,4-trifluoro-1-(2-thienyl)-1,3-butanedione (HTTA) or 4,4,4-trifluoro-1-phenyl-1,3-butanedione (HTPB or BTA), and M represents Pd^{2+} (Do Couto Almeida et al., 2014).

2.2 Bacterial Strains and Culture Conditions

Six *Campylobacter jejuni* strains were used in this study, including five wild-type strains (143, 133, 68, 13, and 144) isolated from chicken carcasses during a surveillance study conducted by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) between October 2017 and July 2018 at federally inspected slaughterhouses (Rodrigues et al., 2021). The isolates, together with their corresponding epidemiological data, were provided by the Laboratório Federal de Defesa Agropecuária do Rio Grande do Sul (LANAGRO-RS) and had been previously characterized as resistant to erythromycin and ciprofloxacin (Buiatte et al., 2023). The sixth strain (IAL2383), a human clinical isolate obtained from the Coleção de Culturas do Instituto Adolfo Lutz (Adolfo Lutz Institute Culture Collection), was included as the reference strain.

All strains were stored at −80 °C in ultra-high-temperature (UHT) milk as a cryoprotectant at the Laboratório de Epidemiologia Molecular (LEPMOL-UFU). For reactivation, frozen stocks

were inoculated into Bolton broth (Oxoid®) supplemented with 5% defibrinated sheep blood (Laborclin®) and incubated at 37 °C for 44 ± 4 h under microaerophilic conditions (5–15% O_2 and 10% CO_2). The enriched cultures were subsequently streaked onto charcoal cefoperazone deoxycholate agar (CCDA; Preston formulation, CM0739, Oxoid®) and incubated under the same conditions. Colony morphology was evaluated according to ISO 10272-1:2017, and Gram staining was performed to confirm the presence of characteristic curved, Gram-negative *Campylobacter* cells. The resulting cultures were used for viability maintenance and all subsequent experiments.

2.3 Determination of Minimum Bactericidal Concentrations of Palladium(II) β-Diketonate Complexes against Planktonic *C. jejuni*

The minimum inhibitory concentration (MIC) of the PdDMSOTTA and PdDMSOBTA complexes was determined using the broth microdilution method in accordance with EUCAST guidelines (EUCAST, 2022, 2024). Twofold serial dilutions of each complex (100–0.78 µg/mL) were prepared in Mueller–Hinton broth (Oxoid®) supplemented with Ca^{2+}, Mg^{2+}, and 5% defibrinated sheep blood (Laborclin®). Bacterial suspensions were prepared from fresh CCDA cultures, resuspended in sterile 0.85% NaCl solution, and adjusted to a turbidity equivalent to a 0.5 McFarland standard.

Assays were performed in sterile 96-well microplates (Sigma-Aldrich®). Briefly, 200 µL of the highest concentration of each complex were added to the first well, followed by twofold serial dilutions across the remaining wells. After dilution, bacterial inocula were added to each well, and the plates were incubated at 37 °C for 44 ± 4 h under microaerophilic conditions.

To determine the minimum bactericidal concentration (MBC), 10 µL aliquots from each well were subcultured onto CCDA agar plates (Oxoid®) and incubated under the same conditions.

The MBC was defined as the lowest concentration of PdDMSOTTA or PdDMSOBTA that resulted in the complete absence of viable colonies on solid medium.

All assays were performed in quadruplicate and included positive controls (medium inoculated with *C. jejuni* without exposure to metal complexes) and negative controls (uninoculated medium).

2.4 Metabolomic Analysis of *C. jejuni* Exposed to the PdDMSOBTA Complex

Metabolomic analysis was conducted using the *C. jejuni* strain 143, selected based on its previously characterized virulence profile (Buiatte et al., 2023). The PdDMSOBTA complex was selected because it exhibited the highest antimicrobial activity among the tested compounds. Bacterial cultures were exposed to PdDMSOBTA (25 µg/mL), ciprofloxacin (10 µg/mL), erythromycin (6.25 µg/mL), peracetic acid (0.4 µg/mL), or left untreated as a control. For metabolite extraction, lyophilized bacterial pellets were resuspended in 1,000 µL of HPLC-grade methanol, vortexed for 5 min, and centrifuged at 13,000 × *g* for 15 min. The supernatants were transferred to clean microcentrifuge tubes, concentrated under vacuum for 30 min, resuspended in 400 µL of methanol, and filtered through 0.22-µm membranes.

Metabolomic analyses were performed using gas chromatography–mass spectrometry (GC–MS; Agilent 7890B GC System coupled to a 5977B MSD) equipped with an Agilent DB-5HT capillary column (30 m × 250 µm × 0.25 µm). High-purity helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. The oven temperature was initially set at 60 °C for 2 min, increased to 280 °C at a rate of 5 °C/min, and held for 30 min. The mass spectrometer operated in full-scan mode (m/z 50–550), with the transfer line set at 240 °C and the quadrupole at 150 °C. The injection volume was 6 µL.

Raw data were processed using MassHunter Qualitative Analysis software (v.10.0). Molecular features were extracted using the Molecular Feature Extraction (MFE) algorithm and converted

to CEF format. The resulting files were imported into Mass Profiler Professional (MPP, v.B.13.1.1; Agilent) for filtering and statistical analysis. Filters included a minimum abundance threshold of 5,000 counts, a retention time tolerance of 0.15 min, and a mass accuracy window of 15 ppm + 2 mDa. Only metabolites present in 100% of samples in at least one experimental group were considered. Metabolite identification and biological interpretation were supported by the KEGG (Kanehisa & Goto, 2000) and MetaCyc (Caspi et al., 2020) databases, enabling pathway annotation and the identification of potential biomarkers associated with treatment-induced biochemical responses.

2.5 Cytotoxicity Assay in Differentiated Caco-2 Cells

The cytotoxicity of the PdDMSOBTA complex was evaluated using a resazurin reduction assay in accordance with ISO 10993-5:2009 (ISO, 2009). Human colorectal adenocarcinoma Caco-2 cells (HTB-37™, ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Cultilab®) supplemented with 20% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% antibiotic-antimycotic solution (Sigma-Aldrich®). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Differentiated cells were exposed to PdDMSOBTA at concentrations ranging from 0.78 to 100 µg/mL for 24 and 48 h. After each exposure period, the culture medium was replaced with phenol red-free DMEM containing 20 µL of Alamar Blue solution (0.6 mM; Sigma-Aldrich®), followed by incubation for 4 h. Cell viability was determined by measuring absorbance at 570 and 600 nm using a microplate reader and calculated according to the manufacturer's instructions.

Cell viability was calculated according to the following equation:

$$cell\ viability\ (\%) = \frac{(O2\ x\ A1)(O1xA2)}{(O2xP1)(O1xP2)} x100$$

where A_1 and A_2 represent the absorbance values of treated cells at 570 nm and 600 nm, respectively; P_1 and P_2 represent the absorbance values of the negative control at 570 nm and 600 nm, respectively; O_1 is the molar extinction coefficient of oxidized Alamar Blue at 570 nm (117,216); and O_2 is the molar extinction coefficient of oxidized Alamar Blue at 600 nm (80,586).

Untreated cells were used as the negative cytotoxicity control (100% viability), whereas cells exposed to 0.05% dimethyl sulfoxide (DMSO) served as the positive cytotoxicity control. According to ISO 10993-5:2009, cell viability below 70% was considered indicative of cytotoxicity. All experiments were performed in three independent biological replicates with five technical replicates per treatment.

2.6 Evaluation of the Biofilm Formation Index of *C. jejuni*

Biofilm formation was evaluated using the Biofilm Formation Index (BFI) as described by Reeser et al. (2007), with minor modifications. Following reactivation, five to ten colonies of each *C. jejuni* strain were inoculated into Mueller–Hinton (MH) broth supplemented with 5% chicken juice (CJ) and incubated at 37 °C for 44 ± 4 h under microaerophilic conditions. Bacterial cultures were harvested by centrifugation, washed three times with sterile saline, and adjusted to an optical density at 600 nm (OD_{600}) of 0.22–0.28 (approximately 2.5×10^7 CFU/mL). Aliquots (20 µL) of the standardized suspensions were transferred to 96-well microplates containing 180 µL of MH broth supplemented with 5% CJ and incubated under the same conditions.

Following incubation, planktonic cells were removed, and the wells were washed three times with sterile phosphate-buffered saline (PBS). The adhered biomass was stained with 1% crystal violet (LaborClin®) for 10 min, washed, air-dried, and solubilized with 200 µL of methanol. Absorbance was measured at 600 nm using a microplate reader (Perlong® DNM-9602). Wells

containing sterile MH broth supplemented with CJ served as negative controls, whereas inoculated wells served as positive controls. All assays were performed in triplicate.

The Biofilm Formation Index (BFI) was calculated according to Naves et al. (2008) using the following equation:

$$BFI = \frac{AB - NC}{SC}$$

where *AB* represents the optical density of the stained adhered biomass, *NC* corresponds to the absorbance of the negative control (medium without microorganisms), and *SC* represents the optical density of the planktonic (suspended) culture.

2.7 Determination of the Minimum Bactericidal Concentration of Palladium(II) β-Diketonate Complexes against Sessile *C. jejuni*

The minimum bactericidal concentration (MBC) of the PdDMSOTTA and PdDMSOBTA complexes against sessile *C. jejuni* cells was determined using preformed biofilms generated as described in Section 2.6. After biofilm formation, the culture medium was carefully removed, and the wells were gently washed with sterile saline to eliminate non-adherent cells. The complexes were prepared by twofold serial dilution (400–3.12 µg/mL) in MH broth supplemented with 5% CJ and added to the biofilms. Plates were incubated for 2 h at 37 °C under microaerophilic conditions.

Following treatment, 10 µL aliquots from each well were transferred to CCDA (Oxoid®) using a sterile pin replicator and incubated at 42 °C for 48 h under microaerophilic conditions. The MBC was defined as the lowest concentration of each complex resulting in the complete absence of bacterial growth on solid medium. All assays were performed in quadruplicate and included untreated biofilms as positive controls and uninoculated medium as negative controls.

2.8 Ultrastructural Evaluation of *C. jejuni* Biofilms by Scanning Electron Microscopy

The ultrastructure of untreated and PdDMSOBTA-treated *C. jejuni* biofilms was examined by scanning electron microscopy (SEM). PdDMSOBTA was selected based on its superior antibiofilm activity demonstrated in the sessile MBC assay.

Biofilms were formed on 5-mm glass beads placed in 24-well plates containing Mueller–Hinton (MH) broth supplemented with 5% chicken juice (CJ), as described in Section 2.6 (Brown et al., 2014). After biofilm formation, samples were treated with PdDMSOBTA at 50 µg/mL, corresponding to the MBC determined for strain 143.

Following treatment, biofilms were fixed overnight at 4 °C in Karnovsky's fixative (2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M phosphate-buffered saline, pH 7.4).

After 24 h, the fixative was removed, and the samples were washed three times with PBS. Post-fixation was performed with 1% osmium tetroxide for 1–2 h at 4 °C, followed by three additional PBS washes. Dehydration was carried out through a graded ethanol series (30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%), with each step lasting 15–20 min, and the 100% ethanol step repeated three times. Samples were dried using a critical point dryer (CPD 030; Baltec, Germany) with liquid carbon dioxide as the transitional fluid. Subsequently, the specimens were sputter-coated with a 20 nm layer of gold using an SCD 050 metallizer (Baltec, Germany) and examined using a Zeiss Supra 55 FEG scanning electron microscope operated at 20 kV.

2.9 Statistical Analyses

Planktonic and sessile MBC data were analyzed using Kaplan–Meier survival analysis. Concentrations above 100 µg/mL for planktonic cells and above 400 µg/mL for sessile cells were treated as right-censored observations, indicating the absence of bacterial elimination at

the highest concentrations tested. All analyses were performed using RStudio software (version 4.4.3).

Metabolomic data were processed using MassHunter Qualitative Analysis software. Comparisons between experimental groups were conducted using unpaired *t*-tests with asymptotic p-value estimation. To control for multiple testing inherent to metabolomic analyses, p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction. Metabolites with adjusted p-values ≤ 0.05 and absolute fold change ≥ 2.0 were considered significantly altered. Only metabolites detected in 100% of the samples were included. Biological interpretation and metabolite identification were supported by the METLIN database (2019), integrated with Agilent Mass Profiler Professional software (version B.13.1.1).

For BFI data, normality was assessed using the Shapiro–Wilk test for each group. Due to non-normal data distribution ($p < 0.05$), comparisons among three or more groups were performed using the nonparametric Kruskal–Wallis test, followed by Dunn's post hoc test with Holm adjustment for multiple comparisons. Pairwise comparisons were conducted using the Mann–Whitney U test. Statistical analyses and graphical outputs were generated using the ggstatsplot package (version 0.13.0).

Cytotoxicity data were analyzed using GraphPad Prism (version 8.0). Comparisons among treatment groups were performed using one-way analysis of variance (ANOVA).

Unless otherwise specified, statistical significance was set at $p < 0.05$.

3 Results

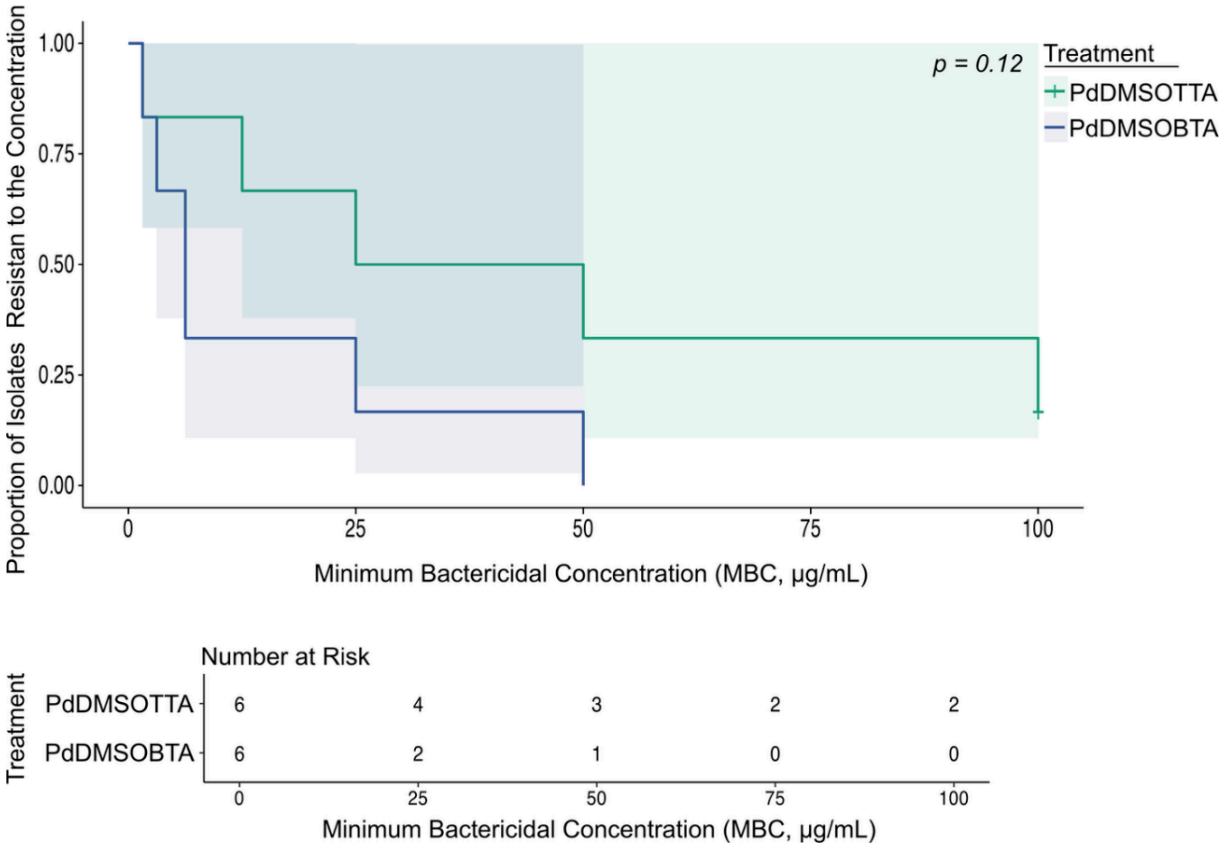
3.1 Determination of the MBC of Palladium(II) β-diketonate complexes against planktonic *C. jejuni*

Considering the overall analysis of the planktonic form, for the PdDMSOBTA complex, the mean MBC observed was 15.36 ± 17.31 µg/mL. The complex exhibited bactericidal activity at low concentrations against isolates 144 (1.56 µg/mL), IAL (3.12 µg/mL), and isolates 143 and 68 (6.25 µg/mL). Higher concentrations were required for the elimination of isolates 133 (25 µg/mL) and 13 (50 µg/mL).

For the PdDMSOTTA complex, the mean MBC was 37.81 ± 35.02 µg/mL, indicating lower bactericidal activity against the evaluated *C. jejuni* isolates. In comparison, the PdDMSOTTA complex demonstrated bactericidal activity at a low concentration only for isolate 144 (1.56 µg/mL), being less active against the other strains. For isolates 68, IAL, 143, and 133, increasing concentrations of 12.5, 25, 50, and 100 µg/mL were required, respectively. Isolate 13 was not eliminated even at the highest concentration tested (100 µg/mL).

The log-rank test did not indicate a statistically significant difference between the treatments ($p = 0.12$). PdDMSOBTA eliminated all isolates (6/6) at concentrations below 50 µg/mL, whereas PdDMSOTTA failed to eliminate one isolate even at the highest concentration tested (Figure 1).

Figure 1: MBC analysis of palladium compounds against planktonic C. jejuni isolates



Kaplan–Meier survival curves for the proportion of *C. jejuni* isolates in the planktonic lifestyle exposed to different concentrations of the palladium compounds PdDMSOTTA and PdDMSOBTA. Isolates not eliminated at the highest concentration tested (>100 µg/mL) were considered right-censored. The reported p-value corresponds to the log-rank test, which evaluates statistical differences between treatments (p = 0.12). The table below shows the number of isolates at risk for each concentration.

3.2 Metabolomics of *C. jejuni* treated with palladium complex and conventional antibiotics

Metabolomic analysis was performed on *C. jejuni* treated with the PdDMSOBTA complex, the conventional antibiotics ciprofloxacin and erythromycin, and the sanitizer peracetic acid. A total of 408 metabolites were initially detected. After applying a 100% frequency filter, only metabolites detected in both samples of each group were retained for statistical analysis.

Metabolites with an adjusted p-value ≤ 0.05 and an absolute fold change ≥ 2.0 were considered significantly altered between treatments. After applying these filters to the comparison between the control group and each treatment, only metabolites meeting these criteria were selected (Table 1).

Table 1: Quantification of metabolites produced by *C. jejuni* that were differentially expressed under each comparative condition tested.

Comparison	Number of metabolites after 100% frequency filter	Number of metabolites after statistical analysis
Control/Palladium	20	5
Palladium/Ciprofloxacin	21	5
Palladium/Erythromycin	22	6
Palladium/Peracetic acid	33	15

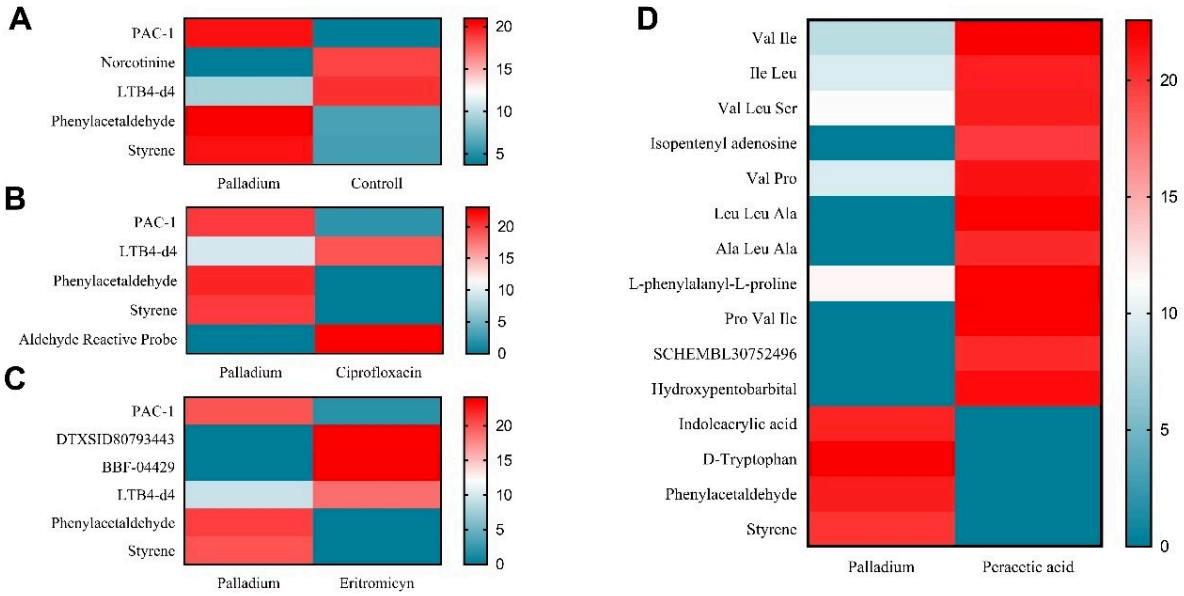
Statistical criteria: unpaired t-test; $p < 0.05$; absolute fold change ≥ 2.

The compounds PAC-1, LTB4-d4, phenylacetaldehyde, and styrene were identified in the comparisons between the palladium/positive control, palladium/erythromycin, and palladium/ciprofloxacin groups. These metabolites are classified, respectively, as a synthetic xenobiotic compound with pharmacological activity, an eicosanoid of the leukotriene class (lipid mediator), an aromatic aldehyde derived from amino acid metabolism (tricarboxylic acid cycle), and a volatile aromatic hydrocarbon of xenobiotic origin.

The metabolite norcotinine, belonging to the class of xenobiotic alkaloids derived from nicotine, was detected exclusively in the comparison between the positive control group and the palladium-treated group (Figure 2A). In contrast, the compounds 2,2′,3,3′,4′,5,6′-heptabromodiphenyl ether, classified as a persistent organic pollutant of the polybrominated diphenyl ether (PBDE) group, and N-demethyl-6-O-methylerythromycin, a semisynthetic macrolide antibiotic and a metabolite of erythromycin, were exclusive to the erythromycin-treated group (Figure 2B). The Aldehyde Reactive Probe, belonging to the class of aldehyde-reactive chemical probes and thiol-derived labeling agents, was identified exclusively in the ciprofloxacin-treated group (Figure 2C).

In the comparison between the palladium- and peracetic acid-treated groups, 15 differentially abundant metabolites were identified, with a predominance of peptide compounds, including dipeptides (Val–Ile; Ile–Leu; Val–Leu–Ser; L-phenylalanyl-L-proline) and tripeptides (Val–Pro; Leu–Leu–Ala; Ala–Leu–Ala; Pro–Val–Ile). Amino acid derivatives associated with the stress response, such as D-tryptophan, were also detected, in addition to purine derivatives (isopentenyl adenosine) and tryptophan metabolites, represented by indoleacrylic acid. The identification of unusual compounds such as thiirane (1,5,5,8-tetramethyl-12-thiabicyclo[9.1.0]dodeca-3,7-diene) and xenobiotic metabolites such as hydroxypentobarbital suggests biodegradation of the medium or interactions with chemical agents. Notably, the only volatile aromatic compounds recurrently detected across all experimental groups were phenylacetaldehyde and styrene, indicating a possible conserved role of these metabolites in the metabolism of *C. jejuni* under different stress conditions (Figure 2D).

Figure 2: Heatmap showing the classification of metabolites in C. jejuni under different conditions



The graph was generated using GraphPad Prism v. 8.0. The heatmap represents the relative abundance profiles of metabolites across different treatment groups: (A) control group versus palladium; (B) palladium versus ciprofloxacin; (C) palladium versus erythromycin; (D) palladium versus peracetic acid. Nomenclature: DTXSID80793443 corresponds to the compound 2,2',3,3',4',5,6'-Heptabromodiphenyl ether (NCBI, 2025b); BBF-04429 corresponds to the compound N-demethyl-6-O-methylerythromycin (NCBI, 2025c); SCHEMBL30752496 corresponds to the compound 1,5,5,8-Tetramethyl-12-thiabicyclo[9.1.0]dodeca-3,7-diene (NCBI, 2025a).

Marked differences in metabolite abundance were observed among the experimental groups, with extremely significant p-values (Supplementary Table 1). In the palladium/control, palladium/ciprofloxacin, and palladium/erythromycin comparisons, PAC-1 and the volatile aromatic compounds phenylacetaldehyde and styrene showed a significant increase (fold change = 20.6, $p \leq 8.78 \times 10^{-5}$) in *C. jejuni* treated with palladium.

In contrast, the metabolite LTB4-d4 showed a reduction associated with palladium treatment, with directional variations depending on the comparison. Among the exclusively detected metabolites, norcotinine showed a reduction in the palladium/control group, whereas the Aldehyde Reactive Probe, identified only in the palladium/ciprofloxacin comparison, also exhibited a marked decrease in abundance. In the comparison with erythromycin, the compounds 2,2′,3,3′,4′,5,6′-heptabromodiphenyl ether and N-demethyl-6-O-methylerythromycin were detected exclusively in this group and showed reduced abundance in palladium-treated *C. jejuni*.

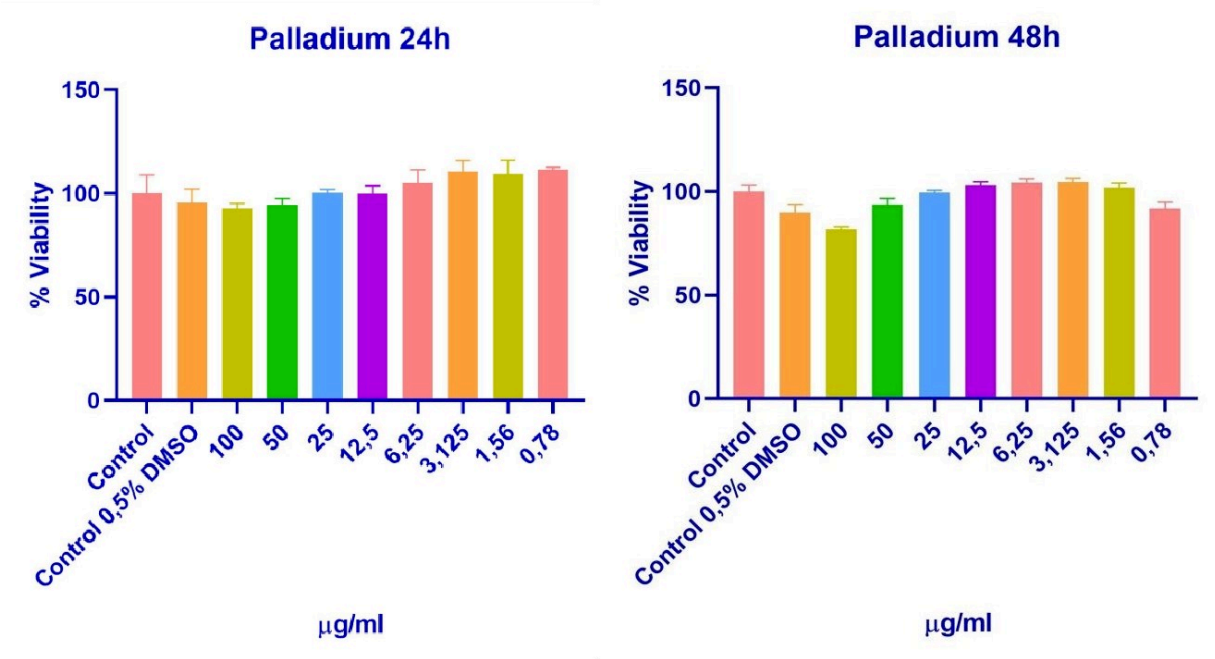
In the comparison between palladium and peracetic acid, a metabolic reorganization was observed. Di- and tripeptides derived from branched-chain amino acids showed a pronounced reduction in the palladium group ($\log_2$ FC ≈ −20 to −22; adjusted $p < 0.01$). These peptides are part of energy metabolism and the biosynthesis of structural cellular components.

The nucleoside isopentenyl adenosine and the xenobiotic hydroxypentobarbital also showed significant reductions in the palladium group (log_2 FC ≈ −19.8 and −21.6, respectively), suggesting an impact on purine/isoprenoid pathways and detoxification processes. The compound 1,5,5,8-tetramethyl-12-thiabicyclo[9.1.0]dodeca-3,7-diene exhibited a marked decrease in the palladium group (log_2 FC ≈ −20.4), whereas compounds related to cellular stress, D-tryptophan and indoleacrylic acid, increased in the palladium group (log_2 FC ≈ 22.6 and 20.7; extremely significant adjusted p-values). The volatile compounds phenylacetaldehyde and styrene also showed increased abundance in the palladium group (log_2 FC ≈ 20–21), following the same pattern observed in the other experimental groups.

3.3 Evaluation of the cytotoxicity of the palladium(II) β-diketonate complex in Caco-2 cells

Due to the superior antibacterial activity demonstrated by the PdDMSOBTA complex, the cytotoxicity of this compound was evaluated in eukaryotic Caco-2 cells differentiated into enterocytes, using the resazurin reduction assay. Cell viability was expressed as a percentage relative to the absolute control (100% viability). A moderate reduction in viability was observed in cells exposed to the compound when compared with the untreated control group. Overall, none of the tested concentrations showed significant cytotoxicity ($p < 0.005$) after 24 h of exposure across all concentrations tested (0.78 to 100 µg/mL), with cell viability remaining above 90%. After 48 h of exposure to the PdDMSOBTA compound, the higher concentrations (50 and 100 µg/mL), as well as the 0.5% DMSO control and the lowest concentration (0.78 µg/mL), showed significant differences ($p < 0.005$) in cell viability relative to the absolute control. However, despite these statistical differences, mean viability values remained close to 80–100% (Figure 3).

Figure 3: Cytotoxicity of the PdDMSOBTA complex in Caco-2 cells



Cell viability (%) of Caco-2 cells treated with PdDMSOBTA at concentrations ranging from 0.78 to 100 µg/mL, evaluated after 24 h (A) and 48 h (B). Bars represent mean values, and lines indicate the standard error of the mean (SEM). Statistical analyses were performed using Dunnett's multiple comparisons test, with the absolute control used as the reference ($\alpha = 0.05$).

3.4 Evaluation of the BFI

All *C. jejuni* isolates evaluated were able to form biofilms, although with different intensities depending on the experimental condition. Overall, the BFI analysis allowed the classification of the strains into strong, moderate, weak, and non-biofilm producers, based on the mean BFI values (Table 2).

Table 2: Classification of *C. jejuni* isolates according to the BFI under different treatment conditions.

Conditions	BFI (N)				
	Strong	**Moderate**	**Weak**	**Non-existent**	**Overall mean**
Control	2.08 ± 0,68 (5)	0.96 (1)	-	-	1.89±0.76 (6)
PdDMSOTTA	1.24 ± 0,02 (2)	0.96 ± 0,06 (4)	-	-	1.05±0.15* (6)
PdDMSOBTA	-	0.74 (1)	0.54 ± 0.16 (2)	0.32 ± 0.01 (3)	0.46±0.18** (6)

BFI classification (mean ± standard deviation) of *C. jejuni* isolates after exposure to palladium complexes. Classification criteria were as follows: BFI < 0.35 (non-existent biofilm); 0.35–0.69 (weak); 0.70–1.10 (moderate); and > 1.10 (strong). N indicates the total number of *C. jejuni* isolates identified in each condition. * indicates a

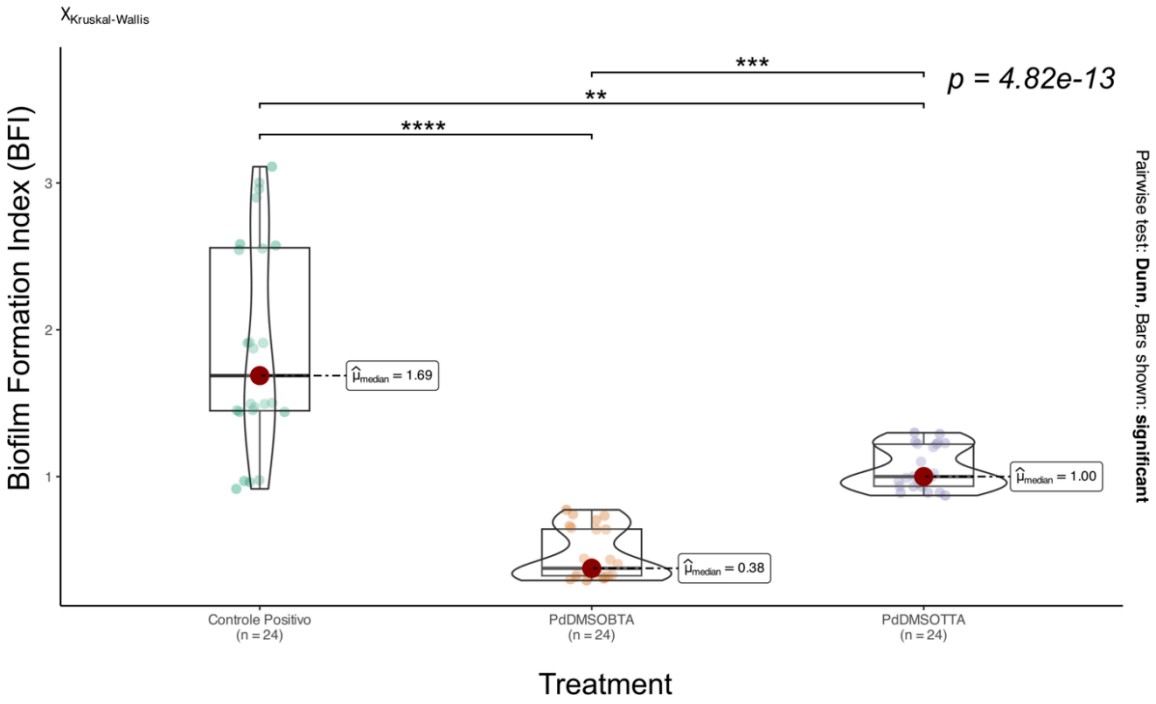
significant difference in comparisons between treatments ($p < 0.005$, Mann–Whitney test); ** indicates $p < 0.0005$ (Mann–Whitney test).

In the control group, a predominance of high-intensity biofilms was observed, with an overall mean BFI of 2.08 ± 0.68 (n = 5), classified as strong, in addition to one isolate classified as moderate (0.96, n = 1). In the presence of the PdDMSOTTA complex, the isolates predominantly formed moderate (0.96 ± 0.06, n = 4) and strong (1.24 ± 0.02, n = 2) biofilms, indicating partial maintenance of adhesion capacity and biomass formation.

In contrast, treatment with PdDMSOBTA promoted greater biofilm reduction. Under this condition, isolates classified as non-biofilm formers (0.32 ± 0.01, n = 3), weak biofilm producers (0.54 ± 0.16, n = 2), and only one isolate classified as moderate (0.74, n = 1) were identified, with no strong biofilms observed.

The overall comparison among experimental conditions (positive control, PdDMSOTTA, and PdDMSOBTA) revealed a statistically significant difference in BFI values (Kruskal–Wallis test, $p < 0.0001$), indicating that the treatments differentially interfered with the adhered biomass of *C. jejuni* (Figure 4).

Figure 4: Global evaluation of the BFI under treatment with palladium complexes



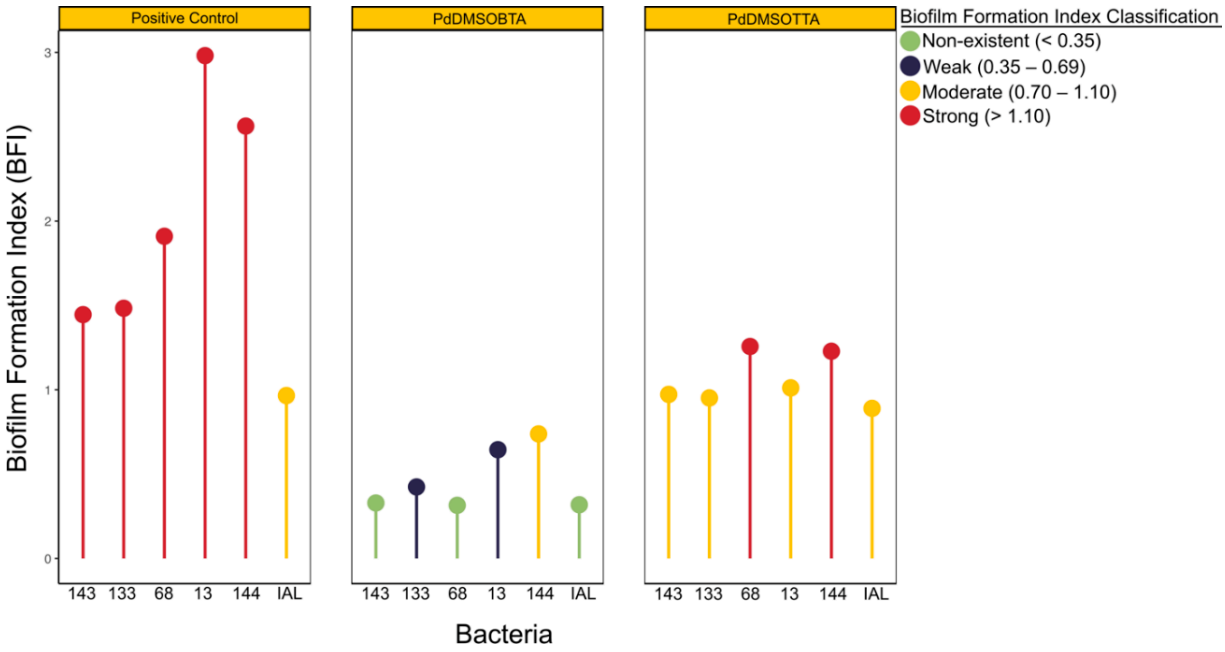
Comparison of the BFI between the Positive Control and the palladium compounds PdDMSOBTA and PdDMSOTTA across different bacteria. Violin plots show the data distribution, with individual data points and the median highlighted. Multiple comparisons were performed using the nonparametric Kruskal–Wallis test followed by Dunn's test, with Holm adjustment for multiple comparisons. $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Upper left corner: global p-value of the Kruskal–Wallis test.

Paired comparisons demonstrated that the PdDMSOBTA complex promoted a marked reduction in adhered biomass in all evaluated isolates. Considering the mean of the isolates, treatment with PdDMSOBTA resulted in an approximate 75% reduction in BFI compared with the positive control (median difference = −1.31; $p = 0.002$; Mann–Whitney test) and a 56% reduction compared with the PdDMSOTTA complex (median difference = −0.62; $p < 0.002$; Mann–Whitney test).

The decrease in BFI induced by the PdDMSOBTA complex led to changes in the phenotypic classification of biofilm formation. Three isolates previously classified as strong (143 and 68) and moderate (IAL) biofilm formers in the positive control were reclassified as non-biofilm

formers after treatment. In addition, isolate 13 showed a reduction in BFI from 3.11 to 0.63, corresponding to a 79% decrease (BFI < 2.48), which represents a transition from a strong biofilm-forming profile to a weak biofilm phenotype (Figure 5).

Figure 5: Evaluation of the BFI in six *C. jejuni* isolates treated with palladium complexes



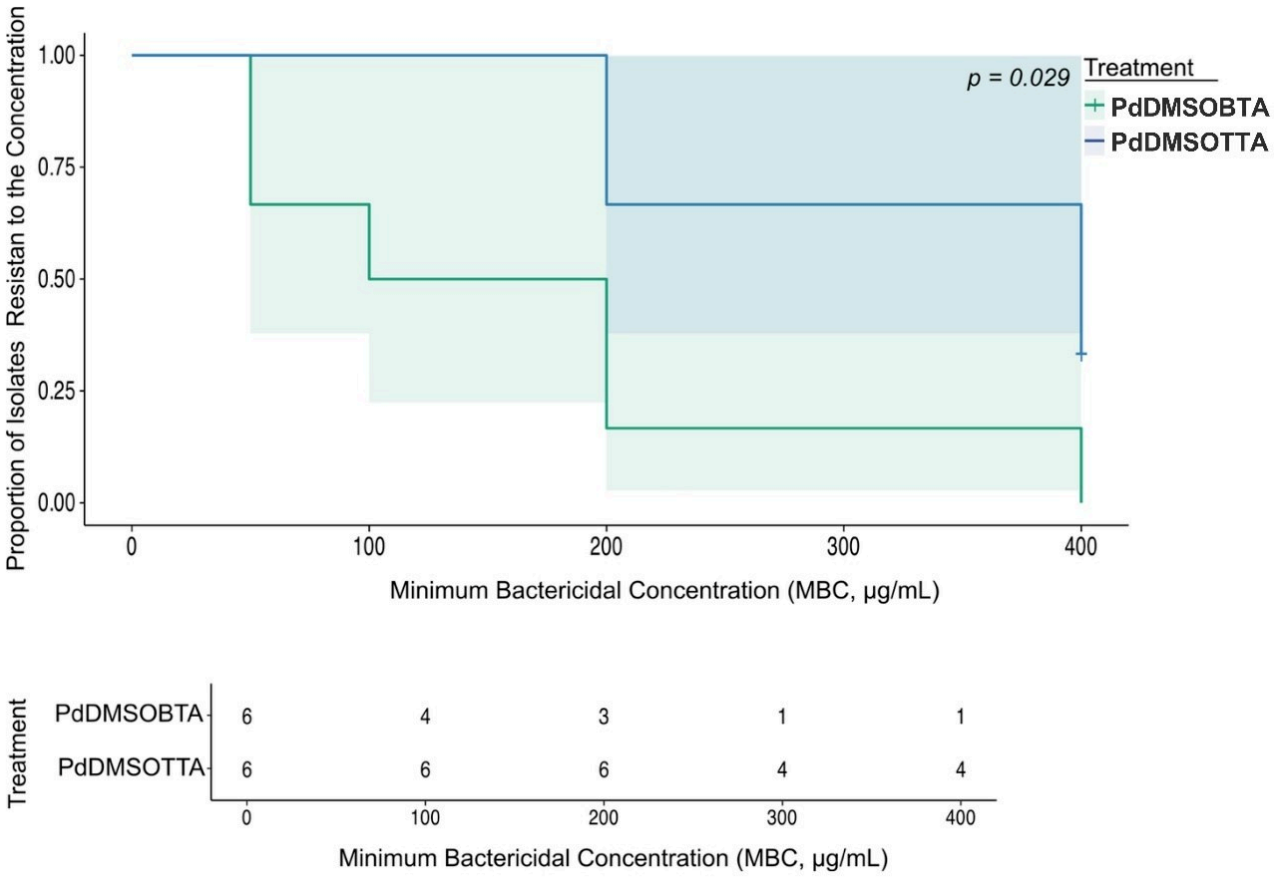
Lollipop-type plot showing the median BFI per isolate and per treatment: Positive Control, PdDMSOBTA, and PdDMSOTTA. Points indicate the median BFI for each isolate, while colors represent biofilm formation categories: non-existent (green), weak (blue), moderate (yellow), and strong (red), according to the classification ranges indicated in the legend.

When evaluating the individual values of *C. jejuni* isolates subjected to treatment with the PdDMSOTTA complex, a reduction in biomass was observed in all strains. This reduction was more pronounced in strains 143, 133, and 13, which were classified as strong biofilm formers in the positive control group. These isolates showed BFI reductions of approximately 0.48 (143), 0.51 (133), and 1.96 (13), resulting in their reclassification into the moderate biofilm category. The smallest variation was observed for the IAL strain, whose BFI decreased slightly from 0.96 ± 0.03 to 0.89 ± 0.01, remaining within the moderate biofilm category.

3.5 Evaluation of the MBC of *C. jejuni* in the sessile form

The MBC was investigated for the six *C. jejuni* isolates against the action of the two palladium complexes. The PdDMSOBTA complex showed superior bactericidal activity, promoting the elimination of 100% of the isolates at concentrations of up to 400 µg/mL. In contrast, the PdDMSOTTA complex exhibited a mortality rate of 66%, since two isolates (144 and 13) were not eliminated even at concentrations higher than 400 µg/mL (Figure 6).

Figure 6: MBC of palladium complexes against sessile C. jejuni bacterial isolates



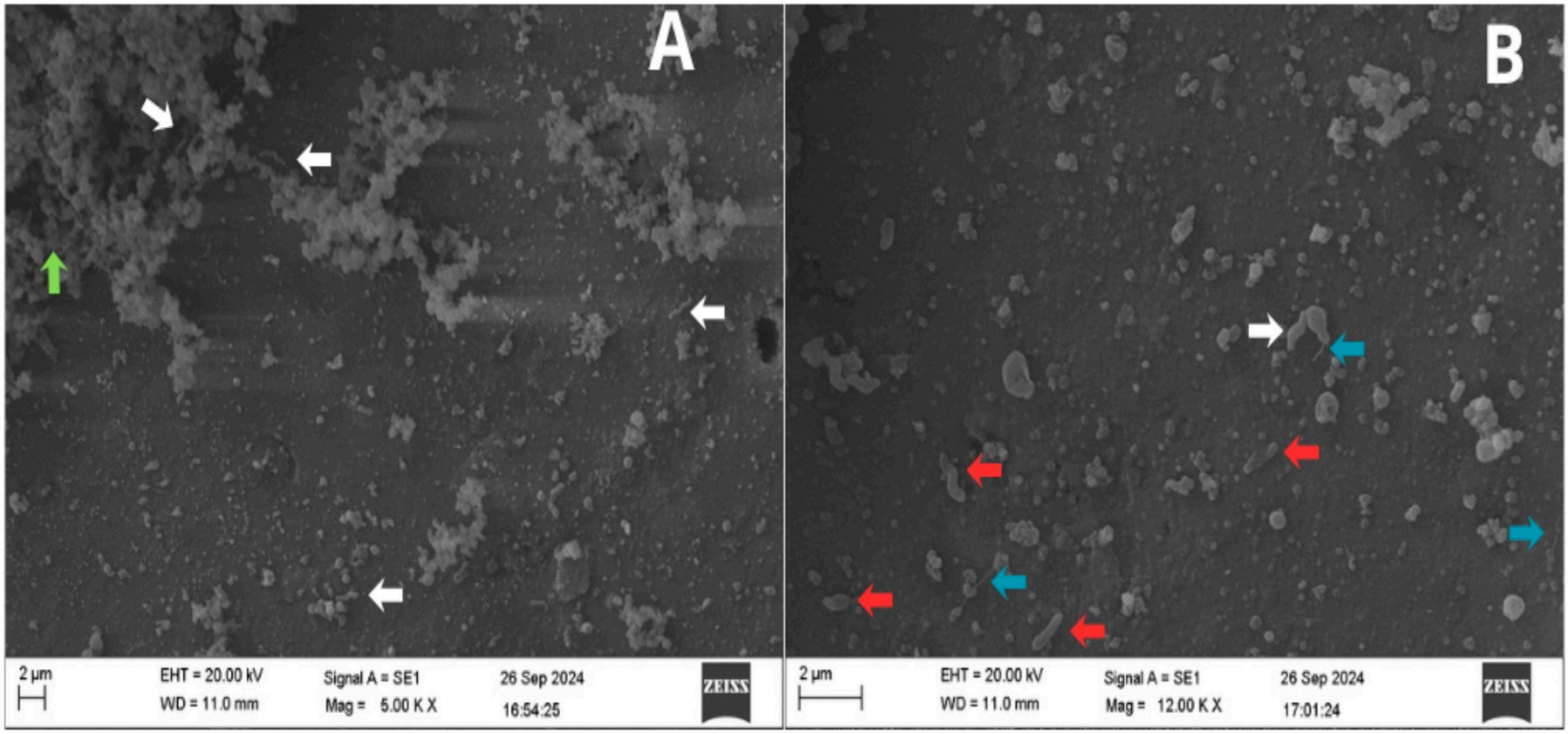
Kaplan–Meier curves represent the proportion of *C. jejuni* isolates resistant/susceptible to increasing concentrations of PdDMSOTTA (green) and PdDMSOBTA (blue). Isolates not eliminated at the highest concentration tested (>400 µg/mL) were treated as right-censored. The log-rank test indicated a significant

difference between treatments (p = 0.029), and the table below shows the number of isolates at risk at each concentration.

3.6 Ultrastructure of sessile *C. jejuni* treated with PdDMSOBTA

SEM analyses allowed visualization of biomass behavior before and after treatment with the palladium(II) β-diketonate complex. In the *C. jejuni* control group, the formation of a mature biofilm was observed, characterized by a three-dimensional structure with an evident matrix, wide pores, expanded architecture, and bacteria exposed on the superficial layer (Figure 7A). In contrast, in the sessile form of *C. jejuni* treated with PdDMSOBTA (50 μg/mL), a sparse biofilm was observed, with matrix degeneration, absence of pores, and exposure of isolated and scarce bacteria, as well as bacteria in the death phase, evidenced by visible bacterial cell disintegration with rupture of the cell membrane (Figure 7B).

Figure 7: SEM images of *C. jejuni* with and without treatment with a metal complex



SEM images of sessile *C. jejuni* (isolate 143). (A) Positive control, with untreated cells, showing the typical morphology of curved to helical bacilli with tapered ends, organized in aggregated structures and surrounded by the extracellular biofilm matrix. (B) Cells treated with the palladium metal complex PdDMSOBTA at a concentration of 50 µg/mL, showing dispersed cells and marked structural alterations. White arrows: *C. jejuni* with intact morphology; green arrow: extracellular matrix; red arrow: *C. jejuni* with disrupted cell membrane, indicating cell death; blue arrow: artifact.

4 DISCUSSION

During slaughter and industrial processing, *C. jejuni* can spread through fecal contamination and cross-contact among carcasses, surfaces, and equipment, contaminating poultry meat and representing a major route of foodborne transmission to humans (Chagneau et al., 2023; Eriksson et al., 2023). Its increasing antimicrobial resistance and biofilm-forming capacity further intensify global public health concerns (Burnham & Hendrixson, 2018; Tram et al., 2020; French et al., 2024; Sung et al., 2024). In this context, metal complexes—particularly palladium-based compounds—have emerged as promising antimicrobial alternatives due to their structural versatility and demonstrated activity against resistant microorganisms and biofilms, with screening by the CO-ADD initiative showing significant antimicrobial activity and low cytotoxicity among palladium complexes (Sovari & Zobi, 2020; Frei et al., 2020).

Our study assessed the palladium complexes PdDMSOBTA and PdDMSOTTA against *C. jejuni* in planktonic and sessile forms. Both compounds are air- and light-stable, soluble in organic solvents, and structurally based on β-diketone curcuminoids coordinated to Pd^{2+} with DMSO as a sulfur ligand, a configuration associated with enhanced bioactive delivery (Do Couto Almeida et al., 2014; Kljun & Turel, 2017; Kamoun et al., 2024). In planktonic bactericidal assays, both complexes were active, with PdDMSOBTA showing greater efficacy (mean CBM = 15.36 ± 17.31 µg/mL) and eliminating all isolates at ≤ 50 µg/mL, whereas PdDMSOTTA displayed a higher mean CBM (37.81 ± 35.02 µg/mL) and failed to eliminate one isolate at 100 µg/mL.

Even for PdDMSOTTA, the CBM values obtained here are markedly lower than those reported for palladium nanoparticles (PdNPs) combined with *Quercus* bioextracts, which showed CBMs of 2,500 µg/mL against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Klebsiella pneumoniae*. No effective activity was observed against *Enterococcus faecalis* and *Escherichia coli*

(MIC and CBM > 5,000 µg/mL), and against *Pseudomonas aeruginosa*, bactericidal effects were restricted to the plant extracts, while PdNPs remained inactive at the tested concentrations (CBM > 5,000 µg/mL) (Coman et al., 2024).

A similar pattern was observed for sessile *C. jejuni*, with PdDMSOBTA showing superior antibiofilm activity. All isolates formed moderate to strong biofilms in the control group, confirming their high adhesion and persistence potential. Exposure to PdDMSOTTA led only to partial biomass reduction, with most biofilms remaining moderate to strong, indicating limited disruption of biofilm structure. In contrast, PdDMSOBTA promoted an average reduction of approximately 75% compared to the positive control and 56% relative to PdDMSOTTA, with the complete absence of strong biofilm classifications.

These findings are consistent with reports showing strong antibiofilm activity of palladium-based formulations. Polymer hydrogels containing $PdCl_2$ combined with sodium alginate, carboxymethylcellulose, and curcumin reduced biofilm formation by up to 77% in *Staphylococcus aureus*, 74% in *Bacillus cereus*, and 66% in *Candida krusei*, an effect attributed to interference with cell adhesion and extracellular matrix integrity (Kamoun et al., 2024). Although conducted with different microorganisms, this pattern aligns with our results, supporting the potentiation of palladium activity when associated with curcuminoid structures.

While PdDMSOTTA exhibited limited efficacy, PdDMSOBTA caused marked biofilm biomass reduction and eliminated 100% (6/6) of sessile isolates at CBM ≤ 400 µg/mL, with a statistically significant difference between treatments (log-rank test, $p = 0.029$). Kaplan–Meier survival analysis and SEM imaging corroborated these findings, revealing destruction of the extracellular matrix, collapse of the three-dimensional biofilm architecture, and severe cellular damage, including membrane rupture, degenerated cells, and dissociated flagella. These effects highlight the strong

antibiofilm potential of PdDMSOBTA, particularly relevant for poultry slaughter environments where *C. jejuni* and *C. coli* form robust biofilms under thermal and oxidative stress (Araújo et al., 2022).

Biofilm formation in *C. jejuni* is closely associated with concerning antimicrobial resistance profiles. In poultry-derived isolates, erythromycin MIC and MBC values ranged widely from 0.25 to 1024 µg/mL in both planktonic and biofilm states (Kostoglou et al., 2024). Moreover, exposure to subinhibitory concentrations of biocides such as peracetic acid, benzalkonium chloride, sodium hypochlorite, iodopovidone, and bis(3-aminopropyl)amine has been shown to increase resistance to multiple antibiotics, including erythromycin, tetracycline, ampicillin, and gentamicin (González-Machado et al., 2025).

Given the strong bactericidal activity of PdDMSOBTA, metabolomic analyses were performed to elucidate palladium-induced stress responses. *C. jejuni* exposed to a sublethal dose of PdDMSOBTA (25 µg/mL) showed metabolic alterations overlapping with those induced by erythromycin and ciprofloxacin. Increased levels of aromatic volatile compounds such as phenylacetaldehyde and styrene, linked to citrate metabolism and aromatic amino acid degradation, suggest metabolic remodeling under stress conditions (Dionisio et al., 2018; Warhurst & Fewson, 1994; Yasuda et al., 2001). Concurrently, a reduction in the lipid mediator LTB4-d4 indicates disruption of lipid metabolism or lipid peroxidation, consistent with stress-induced membrane damage (Golenkina et al., 2025).

The most pronounced metabolic divergence was observed between palladium and peracetic acid treatments. Palladium exposure was associated with increased aromatic metabolites and reduced lipid mediators, branched-chain amino acid–derived peptides, and purine-related metabolites, whereas peracetic acid caused a generalized depletion of peptides ($\log_2$ FC ≈ −20 to −22; adjusted $p < 0.01$) alongside increased stress-related metabolites such as D-tryptophan and indoleacrylic acid (Lee et al.,

2024; Miyakoshi et al., 2022; Hitchcock et al., 2022). These results indicate that PdDMSOBTA more effectively disrupts energy metabolism than conventional sanitizers and antibiotics, supporting its potential as a novel antimicrobial agent.

Cytotoxicity assays in differentiated Caco-2 cells confirmed the safety profile of PdDMSOBTA. Resazurin reduction assays showed >90% cell viability after 24 h at all tested concentrations (0.78–100 μg/mL). After 48 h, minor viability reductions were observed at extreme concentrations, comparable to the DMSO control, with overall viability remaining between ~80–100%. According to ISO 10993-5:2009 criteria, these values indicate no cytotoxicity or only borderline effects, confirming the low cytotoxic potential of PdDMSOBTA within the tested conditions.

5 CONCLUSION

The results of this study demonstrated that palladium(II) β-diketonate complexes, particularly PdDMSOBTA, exhibited consistent and superior antimicrobial performance against *Campylobacter jejuni*, acting effectively on both planktonic cells and mature biofilms. The high bactericidal activity, combined with the ability to significantly reduce adhered biomass, confirms the robustness of the observed effect even under conditions that mimic scenarios of increased bacterial persistence. In addition, metabolomic characterization revealed that exposure to palladium triggered a specific and multifaceted metabolic reorganization, distinct from that induced by conventional antibiotics and oxidizing agents, suggesting a broad mechanism of action associated with interference in central pathways of cellular homeostasis and stress response. Finally, the maintenance of Caco-2 cell viability in the presence of PdDMSOBTA reinforces the favorable biological safety profile of this complex. Taken together, these findings support the potential of PdDMSOBTA as an innovative and promising alternative for strategies aimed at controlling *C. jejuni* in food production–related contexts, particularly

in light of the challenges posed by the sessile lifestyle and resistance to conventional antimicrobial methods.

6 CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 AUTHOR CONTRIBUTIONS

SD, LRMC, CFD, and RTRM contributed to the methodological design of the study. LRMC was responsible for the statistical analyses. CFD contributed to the methodology and performed a critical revision of the manuscript. HOA-S, MMM, LMB, and NS conducted the cell culture experiments and metabolomic analyses. WG provided the palladium compound used in the study. ABGB performed the data analyses. DAR and RTRM conceived the study, coordinated the project, and supervised all stages of the research. All authors contributed to manuscript preparation and approved the final version.

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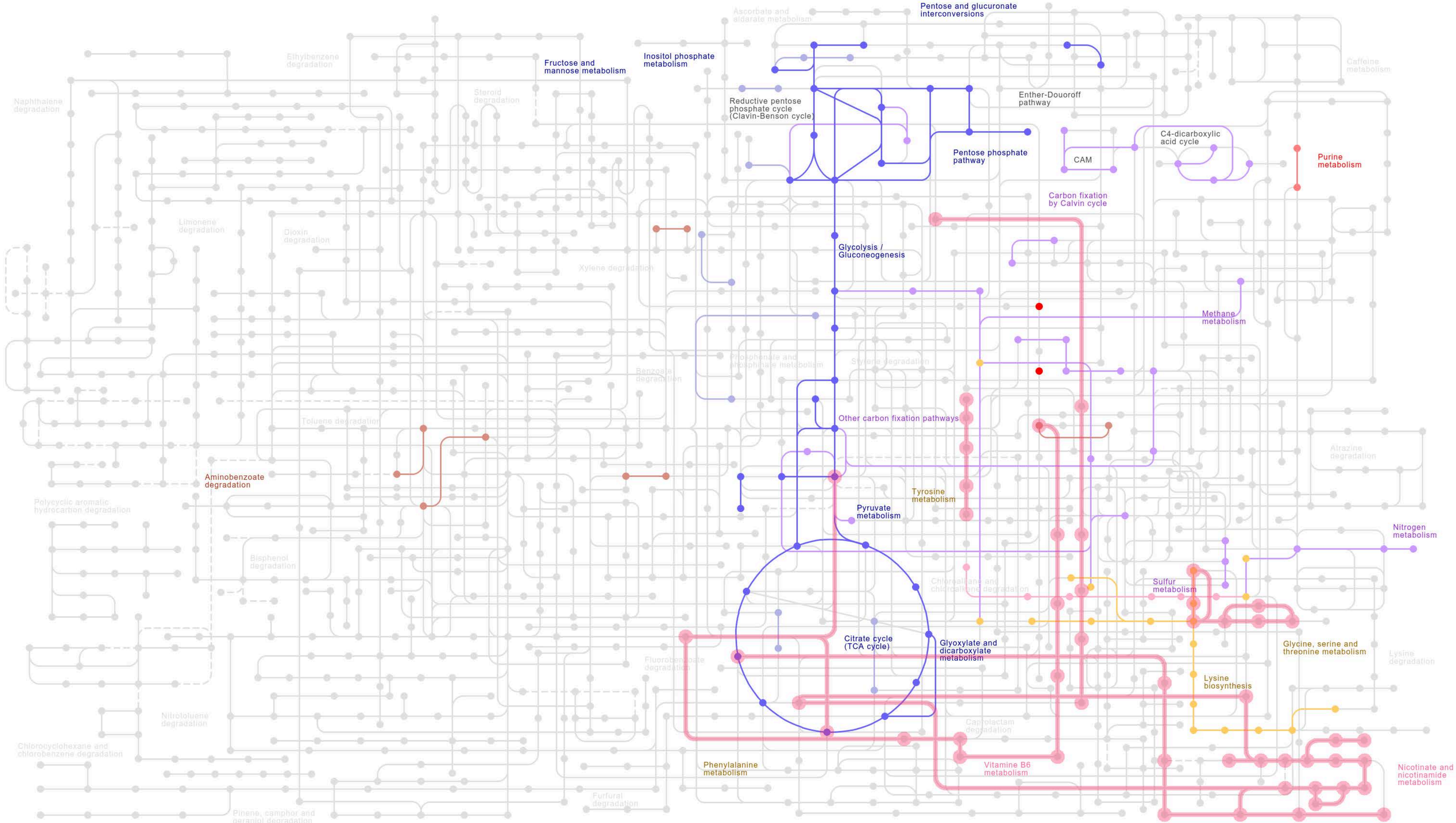

CAPITULO III

STRUCTURAL AND METABOLIC ALTERATIONS IN RESISTANT *Campylobacter jejuni* INDUCED BY PLATINUM(II) B-DIKETONATE COMPLEXES

Antimicrobial Activity and Structural–Metabolic Responses of Resistant *Campylobacter jejuni* to Platinum (II) B-Diketonate Complexes

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Abstract

Campylobacter jejuni is recognized as the leading foodborne gastroenteritis pathogen, exhibiting a high capacity for biofilm formation and increasing antimicrobial resistance. This study evaluated the antimicrobial potential of platinum(II) β-diketonate complexes (PtDMSOBTA and PtDMSOTTA) against six resistant strains in both planktonic and sessile forms, as well as their metabolic effects and cytotoxicity. Antimicrobial activity was determined by minimum bactericidal concentration (MBC). Antibiofilm activity was assessed using the biofilm formation index (BFI) and MBC testing in mature biofilms. Ultrastructural changes were analyzed by scanning electron microscopy (SEM), the metabolomic profile by gas chromatography–mass spectrometry (GC–MS), and cytotoxicity in differentiated Caco-2 cells by the resazurin reduction assay. PtDMSOBTA showed greater bactericidal activity in the planktonic form (12.76 ± 9.21 μg/mL) compared to PtDMSOTTA (51.04 ± 36.87 μg/mL; $p = 0.032$). In the sessile-form MBC assay, PtDMSOBTA eradicated biofilms from all isolates (6/6) at concentrations up to 200 μg/mL, whereas PtDMSOTTA was not effective against all isolates (4/6, $p = 0.0075$). PtDMSOBTA significantly reduced biofilm biomass (74.8%; $p = 0.0313$), while PtDMSOTTA promoted a moderate reduction (29.8%). SEM revealed structural disorganization and reduction of the extracellular matrix after exposure to PtDMSOBTA. Metabolomic analysis identified 408 metabolites, with alterations in pathways related to amino acid metabolism, redox balance, and tryptophan metabolism. Increased cadaverine levels were observed under platinum exposure compared to antibiotics, while LTB4-d4 levels decreased in comparisons involving ciprofloxacin and erythromycin. Both complexes exhibited low cytotoxicity (80–100% viability). PtDMSOBTA emerged as the most promising candidate against *C. jejuni* resistant to macrolides and fluoroquinolones.

Keywords: Antimicrobial resistance; biofilm formation; electron microscopy; metabolomics; poultry industry.

1. INTRODUCTION

Foodborne diseases account for approximately 70% of reported zoonoses, with campylobacteriosis and salmonellosis standing out as the most frequently reported zoonotic diseases in the European Union. Among the etiological agents of campylobacteriosis, *C. jejuni* is notable for its widespread distribution throughout the poultry production chain, which represents the main reservoir associated with human infection (ECDC, 2025).

Although *C. jejuni* infections generally cause self-limiting gastroenteritis, they may progress to severe conditions in high-risk populations, such as pregnant women, older adults, and children, with complications including Guillain–Barré syndrome and bacteremia (ECDC, 2026). In these patients, antibiotic therapy is commonly instituted despite the increasing levels of resistance to quinolones, fluoroquinolones, and β-lactams identified in this bacterium (ECDC/EFSA, 2026). Its ability to acquire and disseminate genetic determinants of resistance through horizontal gene transfer favors the maintenance of multidrug-resistant profiles, which is further intensified by the acquisition of the sessile (Kelbert et al., 2025; Ma et al., 2021).

C. jejuni biofilms possess an extracellular matrix predominantly composed of polysaccharides, proteins, extracellular nucleic acids (eDNA), and lipids (Pavlinjek et al., 2024). In industrial environments, chicken juice (CJ), an exudate released from chicken carcasses during processing and refrigerated storage, coats abiotic surfaces (stainless steel, polystyrene, glass) with a layer rich in proteins and organic matter (Brown et al., 2014). This exudate may also modulate gene expression associated with adhesion, motility, and oxidative stress, promoting increased environmental tolerance, whereby surfaces contaminated with organic residues become favorable niches for the persistence of *C. jejuni*, hindering sanitation procedures and favoring cross-contamination (Man et al., 2024).

Given this scenario, the search for more effective measures to control *C. jejuni* is urgent. Platinum complexes such as cisplatin, carboplatin, and oxaliplatin are widely used in oncology

(Forgie et al., 2022). Platinum is a noble transition metal that stands out in medicinal coordination chemistry due to its versatility in two main oxidation states: Pt(II) and Pt(IV). Pt(II) complexes exhibit a d^8 configuration and predominantly adopt a square planar geometry, which is thermodynamically stable and kinetically labile, allowing ligand substitution in biological environments. As a Pearson soft acid, platinum exhibits high electronic affinity for nitrogen (N)- and sulfur (S)-donor ligands, such as those found in DNA nitrogenous bases and cysteine or methionine residues in proteins, which underlies its biological mechanism of action (Zhang et al., 2022; Royal Society of Chemistry, n.d.). This metal has already demonstrated potent activity against *C. jejuni* in synergism with ciprofloxacin and erythromycin (Lacerda et al., 2022), as well as antifungal (Aly et al., 2025) and antiparasitic activity (Ishmail et al., 2025).

On the other hand, β-diketones are a class of versatile organic compounds widely used in coordination chemistry due to their ability to form stable complexes with metals. These compounds bind to Pt(II) through oxygen atoms, forming complexes with improved solubility in organic solvents (DMSO), lipophilicity, and stability. Such complexes have already demonstrated cytotoxicity in different tumor cell lines (Do Couto Almeida et al., 2014). Therefore, motivated by this promising activity and considering the relevance of *C. jejuni*, we evaluated the efficacy and mechanism of action of Pt(II) complexes coordinated to β-diketones in the control of resistant *C. jejuni* strains, both in planktonic and sessile forms.

2. MATERIALS AND METHODS

2.1. Origin and Storage *C. jejuni* Strains

Six *C. jejuni* strains were used in this study, including five wild-type strains identified as 143, 133, 68, 13, and 144. These strains were previously isolated from chicken carcasses between October 2017 and July 2018. The strains, together with their respective

epidemiological data, were provided by LANAGRO-RS and previously characterized as resistant to erythromycin and ciprofloxacin (Buiatte et al., 2023).

The sixth strain (IAL2383), used as a positive control, was of human clinical origin and obtained from the culture collection of the Instituto Adolfo Lutz. All strains were stored in cryoprotective medium at the Experimental Molecular Epidemiology Laboratory (LEPMOL) of the Universidade Federal de Uberlândia using UHT milk at −80 °C.

For the experimental assays, the strains were initially reactivated by selective enrichment in Bolton broth (Oxoid®) supplemented with 5% defibrinated sheep blood (Laborclin®), followed by incubation at 37 °C for 44 ± 4 h under microaerophilic conditions. Subsequently, enriched cultures were streaked onto charcoal cefoperazone deoxycholate agar (CCDA®) and incubated again under the same temperature, time, and controlled atmosphere conditions (5–15% O_2 and 10% CO_2), using a microaerophilic generation system (Probac®), followed by optical microscopy after Gram staining of the colonies (ISO 10272-1).

2.2. Characterization of Pt(II) Complexes Coordinated to B-diketones

The platinum(II) β-diketonate complexes evaluated in this study were synthesized and provided by the Institute of Chemistry of the Universidade Federal de Uberlândia. The complexes [PtCl(TTA)(DMSO)] and [PtCl(TPB)(DMSO)] follow the general formula [MCl(L)DMSO], in which L corresponds to β-diketone ligands derived from HTTA or HTPB, and M represents Pt^{2+}. Both complexes were obtained through the reaction of cis-[$PtCl_2(DMSO)_2$] with the respective β-diketone ligand under controlled temperature and agitation for 48 h, followed by filtration, washing, and drying.

The products were isolated as yellow solids, with yields of 71% for [PtCl(TTA)(DMSO)], designated PtDMSOTTA, and 40% for [PtCl(TPB)(DMSO)], designated PtDMSOBTA. Elemental analysis, together with infrared (IR) and UV–Vis spectroscopy, as well as molar conductivity measurements, confirmed the successful synthesis

and structural integrity of the complexes. The structural and spectroscopic characterization of these platinum(II) β-diketonate compounds was previously described by Do Couto Almeida et al. (2014).

2.3. Minimum Bactericidal Concentration Test of Pt(II) B-diketone Complexes Against Planktonic *C. jejuni*

The minimum bactericidal concentration (MBC) was determined according to the guidelines of the CLSI M45 document (2016) and EUCAST (2022, 2024), following the standardized broth microdilution antimicrobial susceptibility testing protocol applied to the PtDMSOTTA and PtDMSOBTA complexes. Serial dilutions of the complexes were prepared in the range of 100 to 0.78 μg/mL. Bacterial inocula were obtained from colonies previously cultured on CCDA agar, suspended in sterile saline solution (0.85% NaCl), and adjusted to turbidity equivalent to the 0.5 McFarland standard.

The assays were performed in Mueller–Hinton (MH; Oxoid®) broth supplemented with Ca^{2+}, Mg^{2+}, and 5% defibrinated sheep blood (Laborclin®), using sterile 96-well microplates (Sigma-Aldrich®). Initially, 200 μL of the compound solution were added to the first well (A), while 100 μL of supplemented MH broth were distributed into the remaining wells (B–H). Subsequently, 100 μL were sequentially transferred from well A to well H and homogenized; the final volume was adjusted to 200 μL with supplemented MH broth, followed by incubation at 37 °C for 44 ± 4 h under microaerophilic conditions.

Aliquots of 10 μL from each well were plated onto CCDA agar plates and incubated under the same conditions (37 °C for 44 ± 4 h in a microaerophilic atmosphere). The MBC was defined as the lowest concentration of PtDMSOTTA or PtDMSOBTA capable of completely preventing the growth of viable colonies on solid medium.

All assays were performed in quadruplicate and included positive controls (medium inoculated with *C. jejuni* without exposure to the metallic complexes) and negative controls (non-inoculated medium).

2.4. Metabolomic Analysis of *C. jejuni* Exposed to the PTDMSOBTA Complex

Metabolomic analysis was performed using *C. jejuni* strain 143, selected based on its previously characterized virulence profile (Buiatte et al., 2023). The PtDMSOBTA complex was chosen due to its superior antimicrobial performance. Bacterial cells were subjected to the following treatments: PtDMSOBTA (25 µg/mL), ciprofloxacin (10 µg/mL), erythromycin (6.25 µg/mL), peracetic acid (0.4 µg/mL), and an untreated control (cells not exposed to compounds).

For metabolite extraction, 1,000 µL of analytical-grade methanol were added to the lyophilized bacterial material, followed by vortex homogenization for 5 min. Samples were centrifuged at 13,000 × g for 15 min, and the supernatants were transferred to clean microtubes and subjected to vacuum concentration for 30 min. Subsequently, concentrated extracts were resuspended in 400 µL of methanol and filtered through 0.22 µm membranes.

Metabolomic analyses were conducted by gas chromatography coupled to mass spectrometry (GC–MS; 7890B GC system coupled to a 5977B MSD detector, Agilent), using a DB-5HT capillary column (30 m × 250 µm × 0.25 µm). High-purity helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. The initial oven temperature was maintained at 60 °C for 2 min and increased to 280 °C at a rate of 5 °C/min, with a final hold time of 30 min. The mass spectrometer operated in full scan mode (m/z 50–550), with the transfer line maintained at 240 °C and the quadrupole at 150 °C. The injection volume was 6 µL.

Raw data were processed using MassHunter Qualitative Analysis software (v.10.0), with molecular feature extraction performed by the Molecular Feature Extraction (MFE) algorithm and conversion to CEF format. The obtained files were imported into Mass Profiler

Professional (MPP, v.B.13.1.1; Agilent) for filtering and statistical analysis. Filtering criteria included a minimum abundance of 5,000 counts, retention time tolerance of 0.15 min, and a mass accuracy window of 15 ppm + 2 mDa, considering only metabolites present in 100% of samples from at least one experimental group.

Metabolite identification and biological interpretation were supported by the KEGG (Kanehisa & Goto, 2000) and MetaCyc (Caspi et al., 2020) databases, enabling metabolic pathway annotation and detection of potential biomarkers associated with biochemical responses induced by the treatments. Metabolites showing an adjusted p-value < 0.05 (unpaired t-test) and an absolute fold change ≥ 2.0 between treatments were considered significantly altered. To control for errors associated with multiple comparisons, the Benjamini–Hochberg correction was applied, adopting a significance level of 5%.

2.5. Cytotoxicity Assay in Caco-2 Cells After Exposure to PTDMSOBTA

Cytotoxicity was evaluated using a cell viability assay based on resazurin reduction, conducted according to ISO 10993-5 guidelines (ISO, 2009).

Human colon adenocarcinoma cells (Caco-2; HTB-37™, ATCC) were initially cultured in 25 cm^2 culture flasks at the Nanobiotechnology Laboratory of the Universidade Federal de Uberlândia. Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Cultilab) supplemented with 20% fetal bovine serum, 1% L-glutamine (2 mM), and 1% antibiotic–antimycotic solution containing amphotericin B, penicillin, and streptomycin (Sigma-Aldrich). Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO_2.

After five days of growth, cell subculture was performed by removing the medium, washing the monolayer with unsupplemented DMEM, and adding trypsin–EDTA solution (0.25% trypsin and 0.53 mM EDTA), followed by incubation for 10–15 min at 37 °C and 5% CO_2 to promote cell detachment. Detached cells were collected, centrifuged at 151 × g for 5 min at room temperature, resuspended in fresh medium, and redistributed into new flasks for

expansion. Subsequently, cells were transferred to plates containing 5.0 mL of supplemented DMEM and incubated again under the same conditions.

To obtain confluent monolayers, the cell suspension was seeded into 96-well plates at a density of 1–2 × 10^4 cells per well and incubated for 24 h at 37 °C and 5% CO_2 to allow cell adhesion. To induce the morphological differentiation typical of enterocytes — including microvilli formation, cell polarity, and tight junction development — cultures were maintained for 20 days, with medium replacement every 48 h, as described by Pinto et al.1983.

After the differentiation period, cells were exposed to the PtDMSOBTA metallic complex, selected due to its greater antimicrobial efficacy, at concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/mL. Plates were incubated for 24 and 48 h at 37 °C in a humidified atmosphere containing 5% CO_2. At the end of each exposure period, the culture medium was replaced with phenol red-free DMEM containing 20 µL of Alamar Blue solution (0.6 mM; Sigma-Aldrich). After an additional 4 h incubation period, cell viability was determined by absorbance readings at 570 nm and 600 nm using a microplate reader.

The percentage of cell viability was calculated according to the equation corresponding to the resazurin reduction method:

$$cell\ viability\ (\%) = \frac{(O2\ x\ A1)(O1xA2)}{(O2xP1)(O1xP2)}x100$$

In the equation used, A_1 and A_2 correspond to the absorbance values of treated cells measured at 570 nm and 600 nm, respectively; P_1 and P_2 represent the absorbance values of the negative control at the same wavelengths; O_1 refers to the molar extinction coefficient of oxidized Alamar Blue at 570 nm (117.216); and O_2 corresponds to the molar extinction coefficient of oxidized Alamar Blue at 600 nm (80.586).

Untreated Caco-2 cells were used as the positive control and considered the reference for 100% cell viability. In contrast, cells exposed to 0.05% dimethyl sulfoxide (DMSO)

constituted the negative control and were adopted as 0% viability. Results were expressed as the percentage of cell viability relative to the experimental controls.

According to ISO 10993-5:2009 (ISO, 2009), reductions in viability equal to or greater than 30% (viability < 70%) were interpreted as indicative of cytotoxic effect. Viability values ≥ 80% were classified as non-cytotoxic, whereas intermediate results between 70% and 79% were considered compatible with mild or borderline cytotoxicity.

All experiments were conducted in biological triplicate, with five technical replicates for each evaluated condition.

2.6. Evaluation of the Biofilm Formation Index (BFI) of *C. jejuni*

Five to ten colonies of each strain were transferred to 50 mL Falcon tubes containing 20 mL of MH broth supplemented with 5% chicken juice (CJ). Cultures were incubated under microaerophilic conditions at 37 °C for 44 ± 4 h. Subsequently, cultures were centrifuged at 5,000 rpm, washed three times with sterile saline solution, and adjusted to an optical density at 600 nm (OD_{600}) between 0.22 and 0.28, corresponding to approximately 2.5×10^7 CFU/mL. Aliquots of 20 µL from these standardized suspensions were inoculated into 96-well microplates containing 180 µL of MH broth supplemented with CJ.

In each assay, wells containing only MH broth supplemented with CJ were used as the negative control, whereas wells inoculated with *C. jejuni* suspensions constituted the positive control. Plates were incubated at 37 °C for 44 ± 4 h under a microaerophilic atmosphere.

Biofilm formation was quantified using the crystal violet staining method, as described by Reeser et al., with minor modifications. Briefly, the culture medium was carefully removed, and wells were washed three times with 200 µL of sterile phosphate-buffered saline (PBS) to remove non-adherent cells. After air drying, wells were stained with 150 µL of 1% crystal violet solution (LaborClin®) for 10 min. Excess dye was removed, and the adhered biomass was solubilized with 200 µL of methanol. Absorbance was then measured at 600 nm using a

microplate reader (Perlong DNM-9602). All experiments were performed in triplicate for each strain.

The BFI was calculated according to the following equation:

$$BFI = \frac{AB - NC}{SC}$$

In the equation used, AB corresponds to the optical density of the stained adhered biomass, NC represents the absorbance of the negative control (medium without microorganisms), and SC refers to the optical density of the planktonic (suspended) culture. Therefore, the BFI value expresses the final quantification of the biofilm biomass adhered to the surface (Naves et al., 2008).

2.7. Determination of the MBC of Pt(II) B-diketone Complexes Against Sessile *C. jejuni*

Determination of the MBC against *C. jejuni* biofilms was performed under the same conditions described in Section 2.3. After biofilm formation, the supernatant was carefully removed, and the wells were gently washed with sterile saline solution. Subsequently, the PtDMSOTTA and PtDMSOBTA complexes were added at concentrations ranging from 400 to 3.12 µg/mL, obtained through twofold serial dilutions.

For the procedure, 200 µL of the compound solution were added to the first well (A), while 100 µL of MH broth supplemented with 5% CJ were distributed into the remaining wells (B–H). Serial transfers of 100 µL were performed from well A to well H, with complete homogenization at each step. The final 100 µL were discarded, and the volume in each well was adjusted to 200 µL with supplemented MH broth. Plates containing preformed biofilms were then incubated with the metallic complexes for 2 h under microaerophilic conditions.

After exposure, 10 µL aliquots from each well were transferred using a sterile pin replicator onto CCDA agar plates and incubated under microaerophilic conditions at 42 °C for

48 h. The MBC in biofilms was defined as the lowest concentration of the metallic complex capable of resulting in complete absence of bacterial growth on solid medium.

2.8. Ultrastructural Evaluation of *C. jejuni* Biofilms by Scanning Electron Microscopy (SEM)

The ultrastructure of sessile *C. jejuni* cells from untreated controls and groups treated with the PtDMSOBTA complex was analyzed by scanning electron microscopy (SEM). This complex was selected due to its greater efficacy in biofilm elimination, as demonstrated in the MBC assays.

Biofilm formation was performed in 24-well plates containing 5 mm glass beads previously conditioned in MH broth supplemented with CJ, following the culture conditions described in Section 2.6 (Brown et al., 2014). The PtDMSOBTA complex was applied at a concentration of 50 µg/mL, corresponding to the MBC determined for isolate 143. After treatment, samples were fixed overnight at 4 °C in Karnovsky solution composed of 2.5% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4).

After 24 h, the fixative was removed and the samples were washed three times with PBS. Post-fixation was performed with 1% osmium tetroxide for 1–2 h at 4 °C, followed by three additional washes with PBS. Dehydration was carried out through an ascending ethanol series (30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%), with each step lasting 15–20 min, and the absolute ethanol step repeated three times. Samples were dried using a critical point dryer (CPD 030; Baltec, Germany) with liquid carbon dioxide as the transition fluid. Subsequently, specimens were sputter-coated with a 20 nm gold layer using an SCD 050 sputter coater (Baltec, Germany) and analyzed in a Zeiss Supra 55 FEG scanning electron microscope operating at 20 kV.

2.9. Statistical Analyses

MBC analyses for planktonic and sessile cells were performed using survival analysis based on the Kaplan–Meier method. Concentrations above 100 µg/mL for planktonic cells and above 400 µg/mL for sessile cells were treated as right-censored data, as they indicated absence of bacterial elimination at the highest tested concentrations. Differences were considered statistically significant when $p < 0.05$. All analyses were conducted using RStudio software (version 4.4.3).

Metabolomic data were processed using MassHunter Qualitative Analysis software. Comparisons among experimental groups were performed using unpaired t-tests with asymptotic p-value estimation. To control the multiple comparison problem inherent to metabolomic analyses, p-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction method. Metabolites with adjusted p-values < 0.05 and absolute fold change ≥ 2.0 were considered significantly altered. Only metabolites detected in 100% of samples were included. Biological interpretation and metabolite identification were supported by the METLIN database (2019), integrated into Agilent Mass Profiler Professional software (version B.13.1.1).

For BFI data, normality was assessed using the Shapiro–Wilk test in each group. Since non-normal distribution was observed ($p < 0.05$), comparisons among three or more groups were performed using the non-parametric Kruskal–Wallis test, followed by Dunn's post hoc test with Holm adjustment for multiple comparisons. Pairwise comparisons were conducted using the Mann–Whitney U test. Statistical analyses and graph generation were performed using the ggstatsplot package (version 0.13.0), adopting a significance level of $p < 0.05$. Reductions in BFI were calculated using One-Way ANOVA.

Cytotoxicity data obtained from Caco-2 cells were analyzed using GraphPad Prism software (version 8.0). Comparisons among groups were performed by one-way analysis of

variance (ANOVA), considering differences statistically significant when $p < 0.05$. Before statistical analyses, cytotoxicity data were log_2-transformed to improve variance stabilization.

3. RESULTS

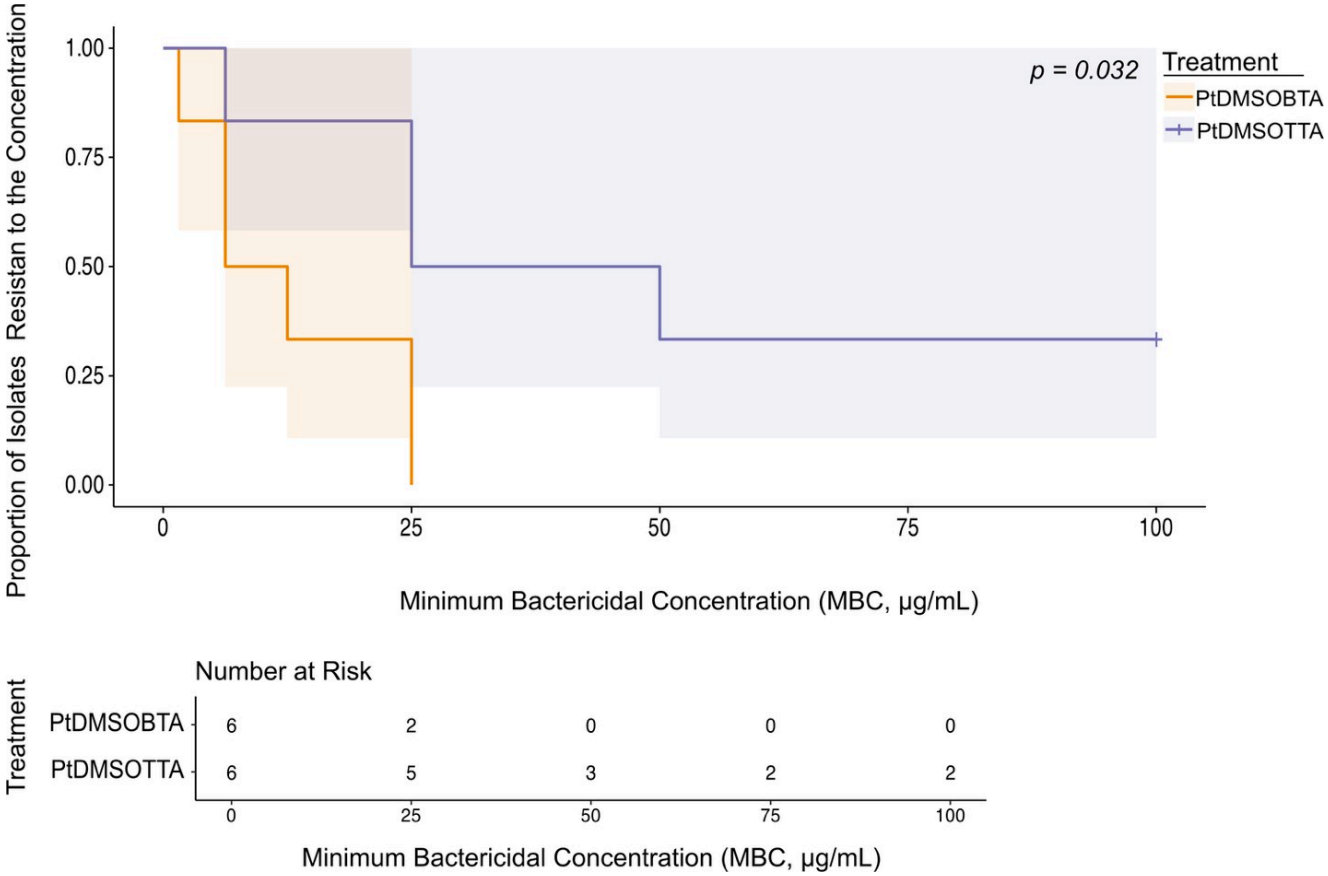
3.1. Determination of the MBC of Pt(II) β-diketone Complexes in Planktonic *C. jejuni* Strains

Considering the global analysis of planktonic cells, the PtDMSOBTA complex showed a mean MBC of 12.76 ± 9.21 μg/mL, demonstrating high bactericidal activity. The compound was effective at low concentrations against isolates 144 (1.56 μg/mL), IAL (12.5 μg/mL), and 143 and 68 (6.25 μg/mL), whereas higher concentrations were required to eliminate isolates 133 and 13 (25 μg/mL).

In contrast, the PtDMSOTTA complex showed a mean MBC of 51.04 ± 36.87 μg/mL, indicating lower bactericidal activity against the evaluated isolates. This compound exhibited bactericidal effect at low concentration only for isolate 144 (6.25 μg/mL), being less active against the remaining strains. For isolates IAL and 143, concentrations of 25 μg/mL were required, and for isolate 68, 50 μg/mL. Isolates 13 and 133 were not eliminated even at the highest tested concentration (100 μg/mL).

The log-rank test indicated a statistically significant difference between treatments ($p = 0.032$). PtDMSOBTA eliminated four isolates at concentrations of 1.56, 6.25 (68 and 143), and 12.5 μg/mL, whereas PtDMSOTTA failed to eliminate two isolates even at the highest evaluated concentration (Figure 1)

Figure 1: Survival analysis of the MBC of platinum compounds against planktonic *C. jejuni* isolates.



Survival curves estimated using the Kaplan–Meier method demonstrating the proportion of bacterial isolates resistant to increasing concentrations of PtDMSOBTA (orange) and PtDMSOTTA (purple). The log-rank test revealed a statistically significant difference between treatments (p = 0.032). Shaded areas correspond to the 95% confidence intervals, and the table below indicates the number of isolates at risk at each concentration level.

3.2. Metabolomics of *C. jejuni* Treated with a Platinum(II) Complex and Conventional Antibiotics

Initially, 408 metabolites were identified. After applying a 100% frequency filter, only metabolites detected in both samples of each group were retained for statistical analysis. Following the application of statistical criteria in the comparison between the control group and each treatment, only metabolites that met these parameters were selected.

Table 1: Quantification of the metabolites produced by *C. jejuni* that showed differential expression under each comparative condition evaluated

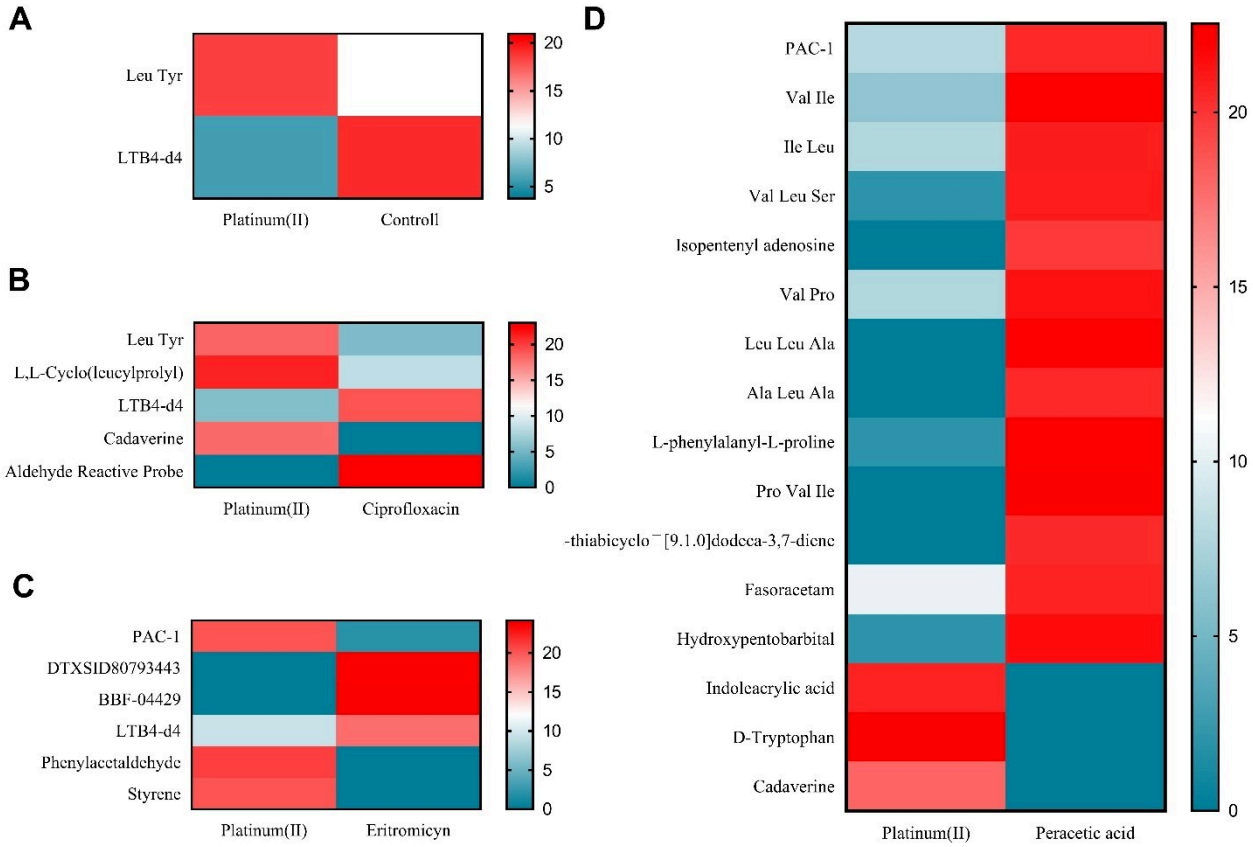
Comparison	Number of metabolites after applying the 100% frequency filter	Number of metabolites after statistical analysis ($p < 0.05$)
Control/Platinum	22	2
Platinum/Ciprofloxacin	23	5
Platinum/Erythromycin	22	4
Platinum/Peracetic acid	34	16

Statistical criteria: unpaired t-test; $p < 0.05$; absolute fold change ≥ 2.

The metabolites Leu-Tyr and LTB4-d4 were identified in the comparisons involving control/platinum and platinum/ciprofloxacin, with LTB4-d4 also detected in the platinum/erythromycin comparison (Figure 2A–C). These compounds correspond, respectively, to a dipeptide derived from branched-chain amino acids and a lipid eicosanoid derived from arachidonic acid. An increase in cadaverine, a biogenic polyamine produced by lysine decarboxylation, was also observed in the platinum/ciprofloxacin, platinum/erythromycin, and platinum/peracetic acid groups (Figure 2D).

In the comparison with ciprofloxacin, in addition to the increase in the Leu-Tyr dipeptide, elevated levels of L,L-cyclo(leucylprolyl) and cadaverine were observed, accompanied by a reduction in LTB4-d4 and the Aldehyde Reactive Probe. The latter belongs to the class of aldehyde-reactive chemical probes used as markers of oxidative stress. Similarly, the comparison with erythromycin showed reduced levels of LTB4-d4 in the platinum treatment, along with decreased levels of 2,2′,3,3′,4′,5,6′-heptabromodiphenyl ether and N-demethyl-6-O-methylerythromycin, organic pollutants derived from erythromycin, whereas cadaverine levels increased, as also observed in the platinum/peracetic acid group.

Figure 2: The graph was generated using GraphPad Prisma v. 8.0.



The heat map shows the relative abundance profiles of metabolites in the different treatment groups: (A) control group versus platinum; (B) platinum group versus ciprofloxacin; The graph was generated using GraphPad Prisma v. 8.0. The heat map represents the relative abundance profiles of metabolites in the different treatment groups: (A) control group versus platinum; (B) platinum group versus ciprofloxacin; (C) platinum group versus erythromycin; (D) platinum group versus peracetic acid.

This latter group (platinum/peracetic acid) revealed the highest number of differentially abundant metabolites, with marked increases in D-tryptophan and indoleacrylic acid, both related to tryptophan metabolism and redox modulation. A predominance of reduced di- and tripeptides derived from branched-chain amino acids was observed, including Val–Ile, Ile–Leu, Val–Leu–Ser, Val–Pro, Leu–Leu–Ala, Ala–Leu–Ala, L-phenylalanyl-L-proline, and Pro–Val–Ile, as well as reductions in isopentenyl adenosine, PAC-1, fasoracetam, and

hydroxypentobarbital, all associated with protein metabolism and amino acid recycling. The main affected pathways are detailed in Supplementary Figure 1.

A reduction in isopentenyl adenosine, a metabolite associated with tRNA modification and translational regulation, was also observed, in addition to compounds such as PAC-1 (Procaspase-Activating Compound 1), an activator of procaspase-3 through zinc ion (Zn2+) chelation and cell death pathways; fasoracetam, a pyrrolidone derivative (5-oxo-D-proline piperidinamide); hydroxypentobarbital, a barbiturate; and the sulfur-containing compound 1,5,5,8-tetramethyl-12-thiabicyclo[9.1.0]dodeca-3,7-diene, an organosulfur compound classified as a thiirane.

3.3. Evaluation of the Cytotoxicity of the Platinum(II) β-diketonate Complex In Caco-2 Cells

PtDMSOBTA exhibited significant toxicity toward Caco-2 cells at higher concentrations (100, 50, and 25 µg/mL) after 24 hours of exposure, with cell viability below 50%. However, viability improved considerably at concentrations lower than 12.5 µg/mL, suggesting lower toxicity within these ranges. Viability values exceeding 100% reflect normal methodological variations and do not indicate a proliferative effect of the compound.

After 48 hours of exposure, cell viability remained low at the highest concentrations but began to improve at concentrations below 6.25 µg/mL, with viability exceeding 50%, indicating low toxicity of the compound at reduced concentrations during this exposure period (Figure 3).

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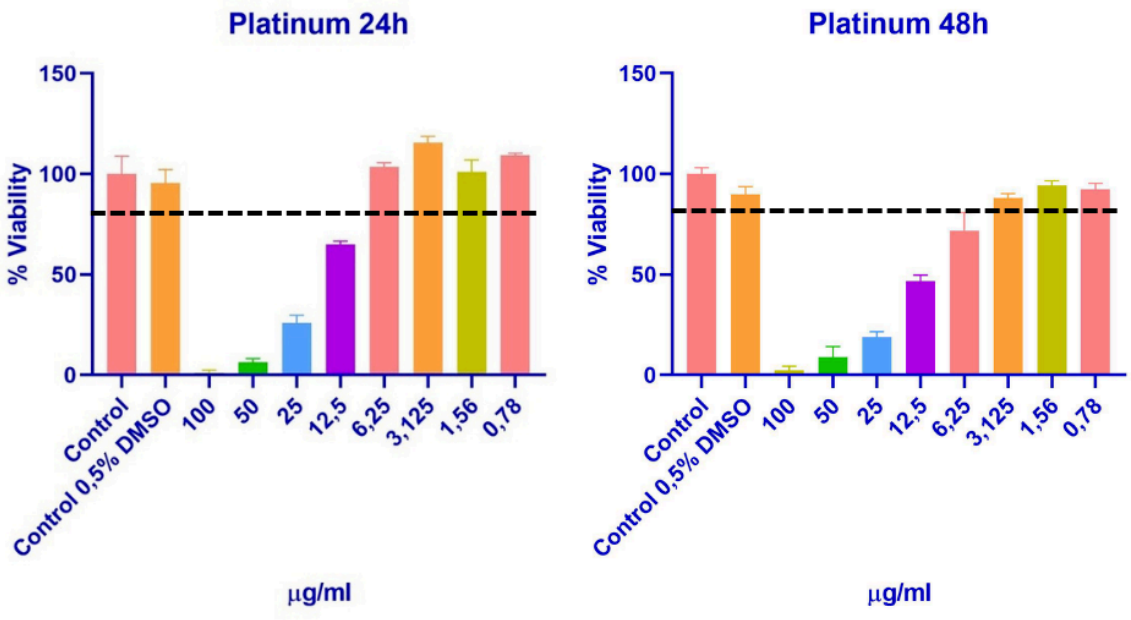
Figure 3: Cytotoxicity of the PtDMSOBTA complex in Caco-2 cells.



Cell viability (%) of Caco-2 cells treated with PtDMSOBTA at concentrations ranging from 0.78 to 100 µg/mL, assessed after 24 h (A) and 48 h (B). The bars represent the mean values, and the lines indicate the standard error of the mean. Statistical analyses were performed using Dunnett's multiple comparison test, with the absolute control as the reference ($\alpha = 0.05$). Viability values below 50% indicate significant cellular toxicity.

3.4. Evaluation of the IFB

All evaluated *C. jejuni* isolates demonstrated the ability to form biofilms, predominantly classified as strong biofilm producers, with a smaller proportion showing moderate and weak biofilm-forming capacity. No isolate was classified as a non-biofilm producer, even after treatment with the metallic complexes (Table 2).

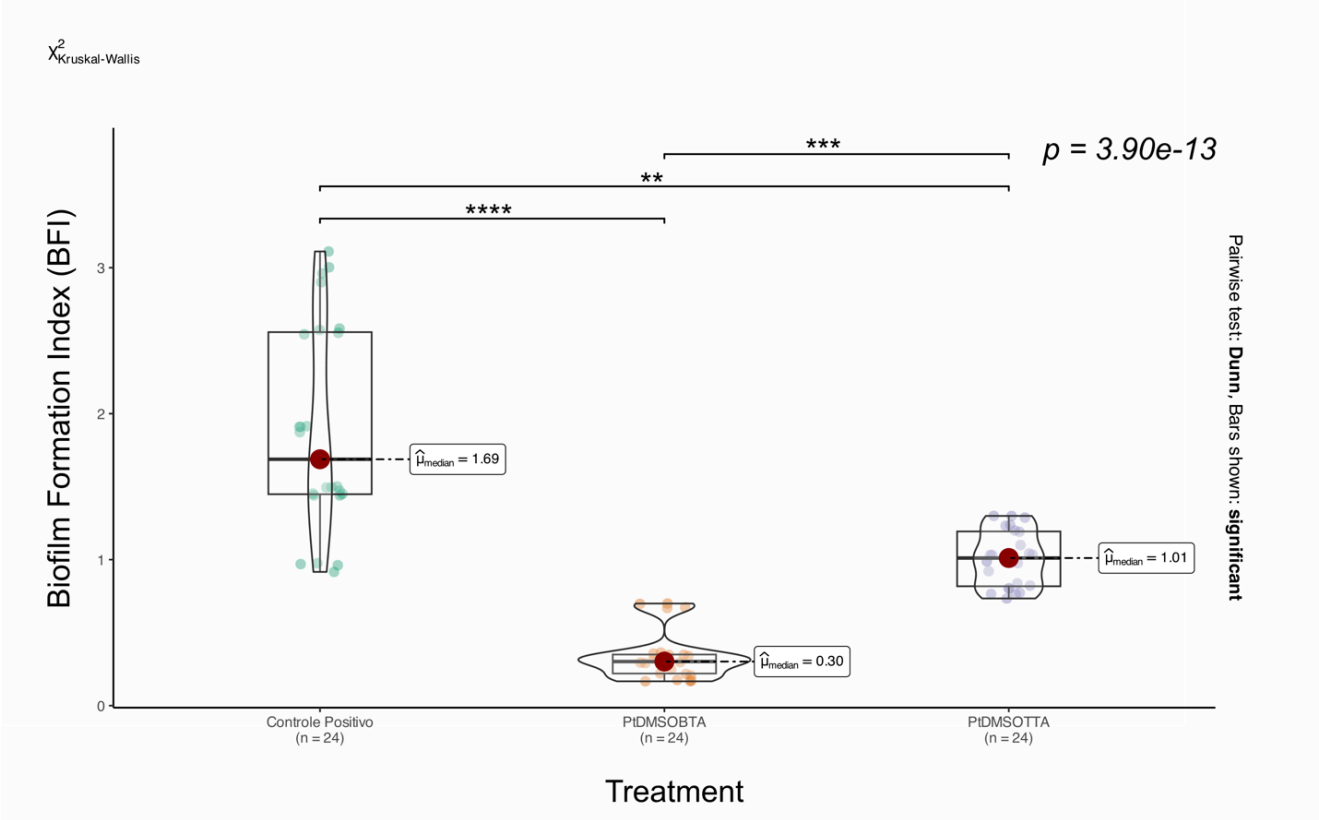
Table 2: Classification of *C. jejuni* isolates according to the IFB under different treatment conditions

Conditions	Strong BFI (n)	Moderate BFI (n)	Weak BFI (n)	Absent BFI (n)	Overall Mean
Control	2.07 ± 0.60 (5)	0.95 (1)	–	–	1.51 ± 0.56 (6)
PtDMSOTTA	1.23 ± 0.63 (2)	0.89 ± 0.11 (4)	–	–	1.06 ± 0.16 (6)
PtDMSOBTA	–	0.51 ± 0.16 (2)	0.25 ± 0.05 (4)	–	0.38 ± 0.13 (6)

(mean ± standard deviation). (n) Number of *C. jejuni* isolates in each classification. The classification criteria were as follows: IFB < 0.35 (no biofilm); 0.35–0.69 (weak); 0.70–1.10 (moderate); and > 1.10 (strong).

It was observed that, in the control condition, most *C. jejuni* isolates (5/6) were classified as strong biofilm producers (2.07 ± 0.60), while only one isolate exhibited moderate biofilm production (0.95). The overall mean BFI in this condition was 1.51 ± 0.56, indicating a high capacity for biofilm formation. After exposure to the PtDMSOTTA complex, a reduction in biofilm formation intensity was observed, with a redistribution of isolates between the strong (1.23 ± 0.63) and moderate (0.89 ± 0.11) categories, with an overall mean of 1.06 ± 0.16. In turn, treatment with PtDMSOBTA resulted in the greatest reduction in biofilm formation, with no isolates classified as strong producers observed. Under these conditions, two isolates were classified as moderate (0.51 ± 0.16) and four as weak (0.25 ± 0.05). The overall average IFB was 0.38 ± 0.13. We observed that both treatments significantly reduced biomass adhesion in *C. jejuni* (Figure 4), especially for the PtDMSOBTA complex.

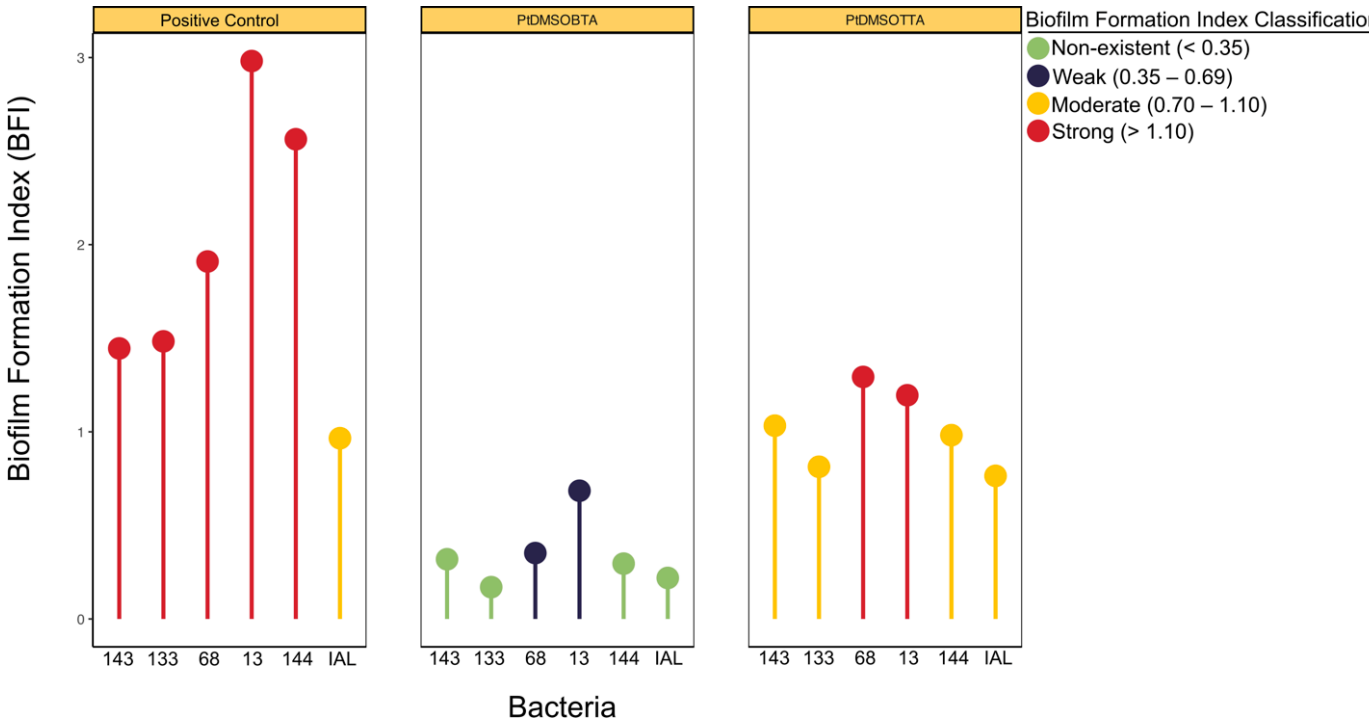
Figure 4: Overall assessment of IFB under treatment with platinum complexes



Comparison of IFB between the Positive Control and the platinum compounds PtDMSOBTA and PtDMSOTTA for six tested strains. Kruskal–Wallis nonparametric test, followed by Dunn's post-hoc test, with Holm's adjustment for correction of multiple comparisons. Significance levels are represented as * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. The overall p-value of the Kruskal–Wallis test is indicated in the graph.

Pairwise comparisons demonstrated that the PtDMSOBTA complex promoted a marked reduction in adhered biomass across all evaluated isolates. Considering the mean BFI values per isolate, treatment with PtDMSOBTA resulted in an approximate 74.8% reduction relative to the positive control (mean difference = 1.13), whereas PtDMSOTTA promoted an approximate 29.8% reduction. Both treatments differed significantly from the positive control ($p = 0.0029$; One-Way ANOVA). These reductions were also observed individually in each isolate, causing phenotypic alterations in biofilm maintenance capacity (Figure 5).

Figure 5: Lollipop plot showing the median BFI for different *C. jejuni* isolates under three conditions: Positive Control, PtDMSOBTA, and PtDMSOTTA



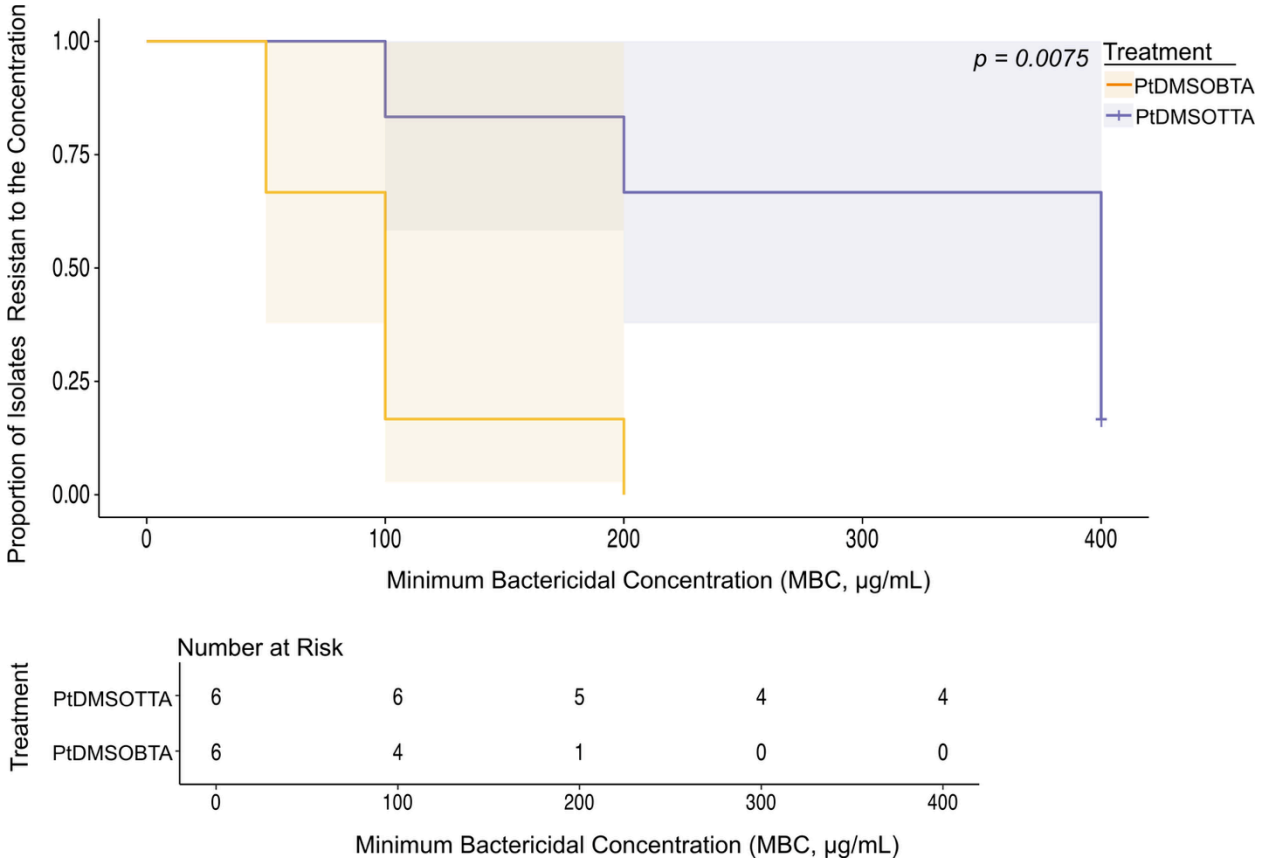
Point colors indicate the biofilm formation category: green for absent, blue for weak, yellow for moderate, and red for strong biofilm formation. This classification is based on the BFI intervals presented in the legend alongside the figure. The figure highlights the variation in biofilm-forming capacity among isolates and treatments.

Isolates originally classified as strong biofilm producers were reclassified after treatment with PtDMSOBTA into weak or non-biofilm-producing categories, considering the individual median BFI values for each isolate. Isolates 144 (2.56), 133 (1.47), and 143 (1.45), initially classified as strong producers in the control group, shifted to the non-biofilm-producing category (0.29, 0.17, and 0.32, respectively). Isolate 13 decreased from 2.99 to 0.68, being reclassified as weak, while isolate 68 decreased from 1.90 to 0.35, positioning at the lower threshold of the weak category. PtDMSOTTA promoted a less pronounced reduction, with predominant reclassification into the moderate category for isolates 144 (0.96), 133 (0.81), and 143 (1.03).

3.5. Evaluation of CBM in Its Sessile Form

The MBC determined for the six *C. jejuni* isolates demonstrated greater bactericidal efficacy for PtDMSOBTA. The values evidenced higher activity of the PtDMSOBTA complex compared to PtDMSOTTA against the evaluated isolates. For PtDMSOTTA, the values ranged from 100 to >400 µg/mL, with the highest values observed for isolates 13 (>400 µg/mL) and 143, 144, and IAL (400 µg/mL). In contrast, PtDMSOBTA exhibited lower CBM values, ranging from 50 to 200 µg/mL, with greater efficacy against isolates 133 and 144 (50 µg/mL). Isolate 13 exhibited the highest resistance profile among those evaluated (Figure 6).

Figure 6: Survival analysis of *C. jejuni* exposed to platinum compounds

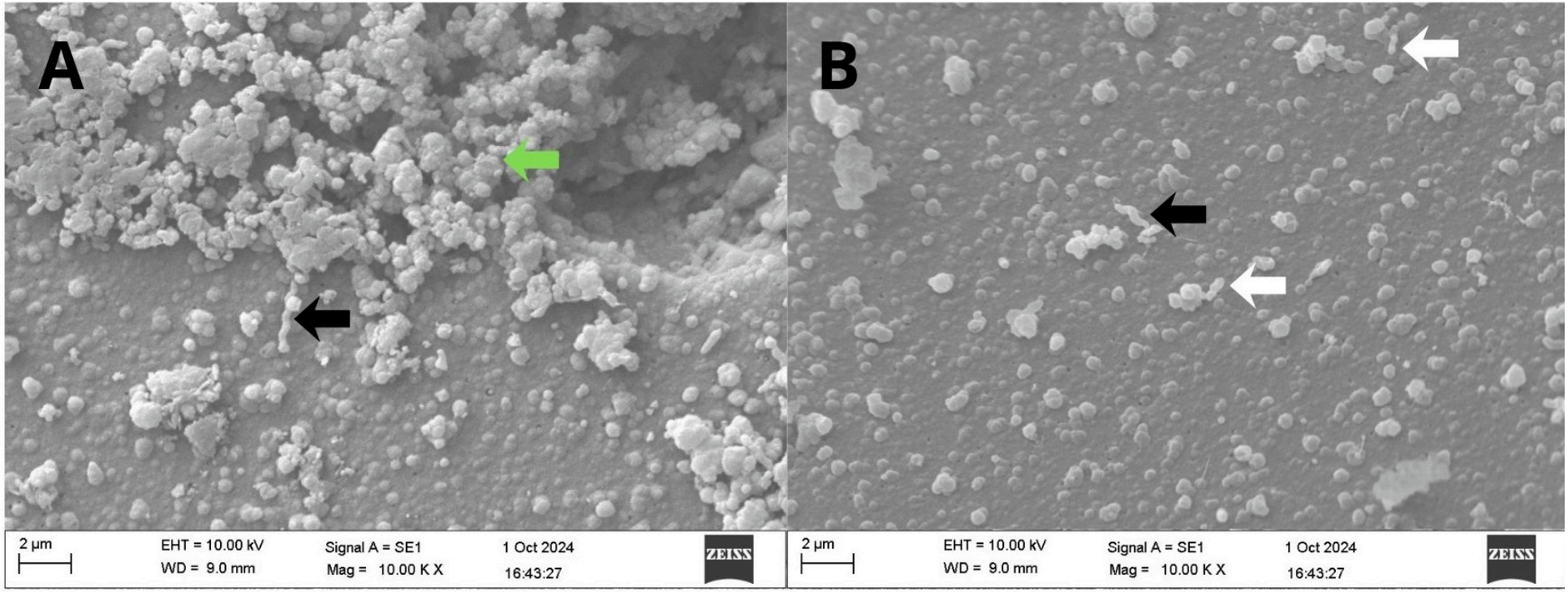


Survival curves estimated using the Kaplan–Meier method demonstrate the proportion of bacterial isolates resistant to increasing concentrations of the compounds PtDMSOBTA (orange) and PtDMSOTTA (purple). The log-rank test indicated a statistically significant difference between treatments (p = 0.0075). The table below shows the number of isolates at risk at each tested concentration.

3.6. MEV of *C. jejuni* Biofilms Treated With PTDMSOBTA

In the control group, the biofilm exhibited a well-structured three-dimensional architecture, with an abundant extracellular matrix and the presence of free-floating bacteria on the surface (Figura 7A). The SEM image of the *C. jejuni* biofilm treated with PtDMSOBTA (50 µg/mL) showed a reduced amount of extracellular matrix. It was also observed that some bacteria had taken on a coccal morphology, which may indicate a transition to the viable but non-cultivable (VNC) state (Figura 7B).

Figure 7: SEM images of sessile *C. jejuni* (isolate 143)



(A) Control showing mature biofilm, a viable *C. jejuni* cell (black arrow), and abundant extracellular matrix (green arrow). (B) Treatment with PtDMSOBTA (50 µg/mL), showing a reduction in the matrix, a viable *C. jejuni* cell (black arrow), and cells with coccoid morphology (VNC) (white arrow).

4. DISCUSSION

The ability of PtDMSOBTA to eliminate resistant *C. jejuni* isolates at relatively low concentrations indicates greater bactericidal activity compared to PtDMSOTTA under the conditions evaluated. In contrast, PtDMSOTTA failed to eliminate two isolates, even at high concentrations. A similar pattern was previously observed against Mycobacterium tuberculosis, in which PtDMSOBTA exhibited an MIC of 24.64 μg/mL, while PtDMSOTTA exhibited an indeterminate MIC greater than 25 μg/mL. Both complexes were described as less active than cisplatin and more active than carboplatin (Do Couto Almeida et al., 2014).

Structural variations in β-diketone ligands alter the antimicrobial activity of Pt(II) complexes through subtle changes in the electronic and steric nature of the ligands. These modifications may affect properties such as thermodynamic stability, kinetic lability, lipophilicity, cellular permeability, and the interaction pattern with biomolecular targets. Similarly, studies involving mono- and binuclear Pt(II) and Pt(IV) complexes containing terminal and bridging nitrito ligands have demonstrated that antimicrobial activity depends not only on ligand structure, but also on the oxidation state of the metal, the complex charge, the number of coordination centers, and the type of metal–ligand bonding. Mixed-valence binuclear complexes, such as PtIV and PtII used in our assays, exhibited greater antimicrobial activity when compared to mononuclear complexes due to the presence of multiple metallic centers, which may enhance the potential for interaction with intracellular molecules (Salishcheva et al., 2020).

Corroborating the influence of structural features on biological activity, Lacerda et al. (2022) evaluated Pt^{2+} complexes containing 5-amino-1,3,4-thiadiazole-2(3H)-thiolate and 1,10-phenanthroline. The platinum complex exhibited significant activity against *C. jejuni* (MIC as low as 0.25 μg/mL) and demonstrated a synergistic effect with ciprofloxacin, whereas the platinum analogue did not display bactericidal activity. These findings indicate that both the

metallic center and the coordinated ligands modulate biological activity and may even influence interactions with antimicrobials targeting DNA replication and integrity.

Most studies have focused on comparative metabolomic analyses involving cisplatin pathways (Poisson et al., 2015) in several tumor cell lines (De Castro et al., 2022; Martins et al., 2021) due to its well-established cytotoxicity in oncology. Although the bactericidal activity of platinum has already been investigated in different formulations, such as nanoparticles, coordinated complexes, and hybrid systems, the application of metabolomics to systematically elucidate the metabolic pathways affected by metals with antimicrobial potential remains relatively underexplored. From this perspective, bacterial exposure to metals may compromise central nutrient acquisition and metabolic pathways, including those related to aspartate and glutamate metabolism, in addition to affecting membrane integrity through the induction of oxidative stress (Belloso Daza et al., 2023; Lacerda et al., 2022).

Our metabolomic data revealed redox imbalance and damage to cellular macromolecules, including DNA and proteins. This primary event tends to increase the demand for repair systems and maintenance of oxidative homeostasis (Rajendran et al., 2020). In this context, activation of the pentose phosphate pathway and modulation of nucleotide metabolism become expected, since these pathways provide NADPH for antioxidant defense and precursors for DNA repair. Alterations in glutathione and other antioxidants, frequently described in the response to platinum, reflect this cellular effort to restore redox balance and preserve structural integrity, suggesting that oxidative stress constitutes one of the central axes of the observed metabolic disturbance (Belloso Daza et al., 2023; Tremaroli et al., 2008).

As a consequence of stress, amino acid metabolism tends to be redirected to meet both energy demands and the need for protein repair. In *C. jejuni*, whose physiology strongly depends on the utilization of amino acids as an energy source, alterations in di- and tripeptides derived from valine, leucine, and isoleucine may indicate intensified catabolism or

redistribution of the intracellular pool of these compounds (Hofreuter, 2014). The predominant reduction of these peptides, especially in the group associated with peracetic acid, is consistent with increased protein turnover or greater metabolic consumption as an attempt to compensate for structural damage and maintain cellular viability under adverse conditions (Yeow et al., 2020).

Alterations in indole metabolites, such as D-tryptophan and indoleacrylic acid, are associated with quorum sensing mechanisms and the regulation of bacterial biofilm formation. Indole can be synthesized, modified, degraded, or metabolized by several bacterial species, including *Escherichia coli*, *Symbiobacterium thermophilum*, *Vibrio cholerae*, *Acinetobacter oleivorans*, *Chromobacterium violaceum*, *Pseudomonas chlororaphis*, and *Serratia marcescens*. In addition to acting as a signaling molecule in bacterial metabolism, microbially derived indole metabolites may also exert effects on the host and have been associated with increased permeability of the gut–blood barrier (Zhang et al., 2021). In our study, increased levels of these metabolites were observed in the PtDMSOBTA-treated group, suggesting possible modulation of bacterial communication pathways under chemical stress, potentially related to biofilm regulation and adaptive mechanisms associated with virulence.

At the post-transcriptional level, the reduction of isopentenyl adenosine (i6A) suggests impairment of tRNA modifications involved in codon-reading stabilization and translational efficiency. i6A is a conserved modification at position 37 (adjacent to the anticodon) of tRNAs that recognize codons initiated by uridine (Yared et al., 2024). A decrease in this modification may affect the fidelity and dynamics of protein synthesis and promote a selective translation state prioritizing essential survival functions. This translational reorganization impacts the expression of enzymes involved in antioxidant responses and polyamine metabolism. Thus, alterations in biogenic amines such as cadaverine may represent a compensatory response to ribosomal instability and oxidative stress, since polyamines participate in membrane

stabilization, protection against reactive species, and modulation of gene expression (Handa et al., 2018; Solmi et al., 2023; Tkachenko et al., 2006).

We also evaluated the biological safety of the platinum complexes considering their possible application as bactericidal agents in the food industry. In this context, Caco-2 cells differentiated into enterocytes are widely used as an in vitro model to simulate the human intestinal epithelium and investigate potential effects associated with the ingestion of compound residues in the final product. High selectivity toward cancer cells has been reported, which may be explained by the affinity for cells with higher metabolic activity and low affinity for normal cells (Gamboa Varela et al., 2014), which may explain the high cytotoxicity in Caco-2 cells observed in our study.

In our assay, we used the PtDMSOBTA complex (0.25 mmol), previously described by Almeida et al. (2014), and observed viability close to 0% at the concentration corresponding to the MBC for *C. jejuni* (100 μg/mL), suggesting compound toxicity under the evaluated conditions. Our values are similar to those reported for five Pt(II) complexes containing Schiff base ligands, which exhibited IC_{50} values ranging from approximately 15 to >100 μg/mL in different cell lines (Caco-2, HeLa, Hep-G2, MCF-7, and PC-3), while maintaining cell viability above 60% (Mbugua et al., 2020).

All *C. jejuni* isolates evaluated were capable of forming biofilms ranging from moderate to strong, a characteristic frequently associated with environmental persistence and increased tolerance to antimicrobial agents. When exposed to platinum complexes, PtDMSOBTA was observed to exhibit greater bacterial elimination capacity, a result consistent with that observed in assays with planktonic cells, suggesting that the higher activity of this complex persists even when bacteria are organized into multicellular structures. Reduction of the extracellular matrix can be observed in SEM images. Studies with platinum-based materials also demonstrate significant antibiofilm activity. For example, platinum nanoparticles were able to significantly

reduce Streptococcus mutans biofilm formation, exhibiting direct adhesion to the bacterial surface and interfering with cell viability during biofilm growth (O'shaughnessy et al., 2020; Sami et al., 2024)

Future studies should include complementary approaches, such as transcriptomic and proteomic analyses, as well as additional cytotoxicity assays and more complex experimental models to better understand biological safety and the molecular mechanisms involved. These findings also suggest that further research should explore a series of platinum complexes with systematic structural variations in the β-diketone ligands, with the aim of identifying structural features associated with greater bactericidal activity and improved biological selectivity.

5. CONCLUSION

The platinum(II) β-diketone complex PtDMSOBTA was able to eliminate *C. jejuni* at low concentrations in its free form, as well as when treating biofilm after it had formed. Compared to PtDMSOTTA, the compound demonstrated greater bactericidal activity, indicating that the ligand structure influences the biological activity of platinum complexes. Metabolomic analyses indicated that exposure to PtDMSOBTA promotes metabolic changes associated with redox balance, amino acid metabolism, translation regulation, and bacterial signaling molecules. These results suggest that the mechanism of action involves interference with processes related to cellular homeostasis, rather than a single specific molecular target. Cytotoxicity assays in differentiated Caco-2 cells indicated low cell viability at concentrations associated with bactericidal activity (100 μg/mL). We can conclude that the platinum compounds tested in this study are not very promising for application in the food industry, since high concentrations are required to eliminate biofilms. However, the PtDMSOBTA compound may represent a promising candidate for the development of strategies aimed at controlling *C.*

jejuni, particularly applicable to environmental control, where biofilm formation remains a challenge.

6. CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7. AUTHORS' CONTRIBUTIONS

SD, LRMC, CFD, and RTRM contributed to the methodological design of the study. LRMC was responsible for the statistical analyses. CFD contributed to the methodology and performed a critical review of the manuscript. HOA-S, MMM, LMB, and NS conducted the cell culture experiments and metabolomic analyses. WG provided the platinum compound used in the study. ABGB performed the data analysis. DAR and RTRM conceived the study, coordinated the project, and supervised all stages of the research. All authors contributed to the preparation of the manuscript and approved the final version.

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