UNIVERSIDADE FEDERAL DA GRANDE DOURADOS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA E TECNOLOGIA AMBIENTAL

RENATA PIRES DE ARAÚJO

OCORRÊNCIA, SOROTIPO, SUSCEPTIBILIDADE ANTIMICROBIANA, IDENTIFICAÇÃO DE INTEGRONS E SEQUENCIAMENTO DE *Salmonella* spp. ISOLADA EM PISCICULTURAS

Dourados -MS
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Dedico a Deus!

A Minha Família!

Ao meu companheiro de vida e amor da minha vida!

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Fracasso não é derrota, é o dom de recomeçar!

Chico Xavier

LISTA DE ABREVIATURAS E SIGLAS

ACR	Acriflavina
	Acriflavine
AFLP	Polimorfismo de Comprimento de Fragmento Amplificado
	Amplified Fragment Length Polymorphism
AMC	Amoxacilina/Ácido Clavulânico
	Amoxicillin/Clavulanic Acid
AME	Enzimas modificadoras de aminoglicosídeos
	Aminoglycoside modifying enzymes
AMG	Aminoglicosídeos
	Aminoglycosides
AMP	Ampicilina
	Ampicillin
ATCC	Coleção de Microrganimos Americana
	American Type Culture Collection
ATP	Adenosina Trifosfato
	Adenosine Triphosphate
AV	Avibactam
	Avibactam
BNKC	Cloreto de Benzalcônio
	Benzalkonium Chloride
BPW	Água Peptonada
	Buffered Peptone Water
C	Cloranfenicol
	Chloramphenicol
CA	Ácido Clavulânico
	Clavulanic Acid
CAT	Cloranfenicol Acetiltransferases
	Chloramphenicol Acetyltransferases
CAZ	Ceftazidima
	Ceftazidime
Cb	Carbapenem

	Carbapenem
CDC	Centros de Controle e Prevenção de Doenças
	Centers for Disease Control and Prevention
CEUA	Comissão de Ética no Uso de Animais
	Ethics Commission on the Use of Animal
CIP	Ciprofloxacina
	Ciprofloxacin
CLP	Cloranfenicol
	Chloramphenicol
CLSI	Instituto de Normas Clínicas e Laboratoriais
	Clinical and Laboratory Standards Institute
CN	Gentamicina
	Gentamicin
Cp	Cefalosporina
	Cephalosporin
CRO	Ceftriaxona
	Ceftriaxone
CTX	Cefotaxima
	Cefotaxime
CV	Cristal Violeta
	Crystal Violet
DNA	Ácido Desoxirribonucléico
	Deoxyribonucleic Acid
DOX	Doxorrubicina
	Doxorubicin
DTA	Doença Transmitida por Alimento
	Foodborne Disease
E	Cefalosporina de Espectro Expandido
	Extended-Spectrum Cephalosporin
EGM	Elementos Genéticos Móveis
	Mobile Genetic Elements
ESBL	Beta-Lactamase de Espectro Estendido
	Extended Spectrum Beta-Lactamases

FAO	Organização das Nações Unidas para a Alimentação e a Agricultura
	Food and Agriculture Organization of the United Nations
FDA	Administração de Alimentos e Medicamentos
	Food and Drug Administration
FOX	Cefoxitina
	Cefoxitin
FSIS	Serviço de Inspenção e Segurança Alimentar
	Food Safety and Inspection Service
H_2S	Gás sulfídrico
	Hydrogen Sulfide
ISO	Organização Internacional de Normalização
	International Organization for Standardization
LDC	Lisina descarboxilase
	Lysine Decarboxylase
LPS	Lipopolissacarídeo
	Lipopolysaccharides
M	Monobactam
	Monobactam
MAC	Macrolídeos
	Macrolides
MB	Azul de metileno
	Methylene Blue
MBLs	Metalo-β-lactamases
	Metallo-β-lactamases
MDR	Mutidroga resistente
	Multidrug Resistance
MLEE	Eletroforese de Enzimas Multilocus
	Multilocus Enzyme Electrophoresis
MLS	Macrolídeos, Lincosaminas e Estreptogramina
	Macrolide, Licosamide and Streptogramin
MLST	Tipagem de Sequência Multilocus
	Multilocus sequence type

MS	Mato Grosso do Sul
	Mato Grosso do Sul
NA, NAL	Ácido Nalidíxico
	Nalidixic Acid
NARMS	Sistema de Monitoramento de Resistência Antimicrobiana Nacional
	National Antimicrobial Resistance Monitoring System
NOR	Norfloxacina
	Norfloxacin
NOV	Novobiocina
	Novobiocin
NTS	*Salmonella* Não-Tifóide
	Nontyphoidal Salmonella
ODC	Ornitina descarboxilase
	Ornithine Decarboxylase
OMS	Organização Mundial de Saúde
	World Health Organization
P	Penicilina
	Penicillin
PCR	Reação em Cadeia da Polimerase
	Polymerase Chain Reaction
R6G	Rodamina 6G
	Rhodamine 6G
RV	Rappaport-Vassiliadis
	Rappaport-Vassiliadis
S	Estreptomicina
	Streptomycin
SDS	Dodecil Sulfato de Sódio
	Dodecyl Sodium Sulfate
SX	Sulfonamida
	Sulfonamide
ST	Tipo de Sequência
	Sequence Type
TE, TET	Tetraciclina

	Tetracycline
Tn	Transposon
	Transposon
TT	Tetrationato
	Tetrathionate
USDA	Departamento de Agricultura dos Estados Unidos
	U.S. Department of Agriculture
VM	Vermelho de Metila
	Methyl Red
VP	Voges-Proskauer
	Voges-Proskauer
W	Trimethoprim
	Trimethoprim
WHO	Organização Mundial da Saúde
	World Health Organization
XLD	Xilose-lisina-tergitol
	Xylose-lysine-tergitol

LISTA DE SÍMBOLOS

Símbolo	Significado
β	Beta
cm	Centímetros
°C	Graus Celsius
h	Horas
mg/L	Miligrama/Litro
mL	Mililitro
µm	Micrometro
%	Porcentagem
>	Maior que

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Ocorrência, Sorotipo, Susceptibilidade Antimicrobiana, Identificação de Integrons e Sequenciamento de *Salmonella* spp. isolada em Pisciculturas

RESUMO

Aquicultura tem se expandido nas últimas décadas fornecendo alimento de alto valor nutricional, e acompanhado com essa expansão, surgem alguns problemas sanitários, como a ocorrência de *Salmonella* spp. Esta bactéria é o principal microrganismo associado a doença transmitida por alimentos no mundo, e a cadeia produtiva de aves, bovinos e suínos são os principais responsáveis por sua disseminação. No entanto, na cadeia produtiva de peixes também existem alguns relatos de isolamento de *Salmonella*. Neste sentido, nosso objetivo foi avaliar a presença, o sorotipo, o perfil de susceptibilidade antimicrobiana e genes de resistência de *Salmonella* spp. em pisciculturas na região da Grande Dourados/MS. O estudo foi conduzido em três pisciculturas onde a primeira fazenda trabalha com monocultivo de Lambari (*Astyanax lacustris*), a segunda com policultivo com quatro espécies nativas: Tambaqui (*Colossoma macropomum*), Pacu (*Piaractus mesopotamicus*), Dourado (*Salminus brasiliensis*) e Patinga (*Piaractus mesopotamicus*) e a terceira fazenda com monocultivo de Tilápia do Nilo (*Oreochromis niloticus*). As amostragens ocorreram de outubro de 2017 a maio de 2018, onde noventa amostras, incluindo vinte e quatro amostras de peixe, quarenta e oito de água e dezoito de biofilme epilítico, foram analisadas e treze *S. enterica* foram isoladas, identificadas por Reação em Cadeia da Polimerase e sorotipadas como *S.* Anatum, *S.* Minnesota, *S.* Panama e dois isolados classificados apenas como *Salmonella enterica* subsp. *enterica*. As maiores taxas de resistência foram às sulfonamidas, trimetoprim, tetraciclina e estreptomicina. A resistência múltipla aos antibióticos foi confirmada e integrons de Classe 1 foram detectados. A caracterização genotípica da *Salmonella* Panama foi realizada e o genoma bacteriano foi sequenciado em uma plataforma Illumina NextSeq® e *de novo* montado usando CLC Genomic Workbench 12. A cepa *Salmonella* Panama pertenceu ao ST2041 e apresentou genes de resistência a aminoglicosídeos, bem como genes que codificam bombas de efluxo de múltiplas drogas e aqueles que conferem resistência a biocidas e metais pesados. Além disso, a cepa abrigava genes que codificam diversos fatores de virulência. Assim, a presença de *Salmonella* na cadeia produtiva de peixe demonstra a ampla distribuição e adaptação deste microrganismo. O perfil de resistência antimicrobiana e características genotípicas alertam para o cuidado em relação ao uso de agentes antimicrobianos para se evitar disseminação de genes de resistência. A utilização de estações de monitoramento, como biofilmes epilíticos pode ser utilizada como sentinela na detecção de *Salmonella* em tanques em fazendas peixe. É sabido que a transmissão de *Salmonella* spp. normalmente ocorre para o homem através do consumo de alimentos contaminados. Portanto, o seu mapeamento na cadeia produtiva de peixes fornece subsídios para o desenvolvimento de medidas de controle de surtos epidêmicos.

Palavras-chave: Salmonelose. Biofilmes epilíticos. Animais de produção.

Occurrence, Serotype, Antimicrobial Susceptibility, identification of Integrons and Sequencing of *Salmonella* spp. isolates in Fish Farms

ABSTRACT

Aquaculture has expanded in recent decades providing food with high nutritional value and accompanied by this expansion, some health problems arise, such as *Salmonella* spp. This bacterium is the main microorganism associated with foodborne disease in the world, and the poultry, cattle and swine production chain are mainly responsible for its spread, however in the fish production chain there are some reports of isolation of *Salmonella*. In this sense, our objective was to evaluate the presence, serotype, antimicrobial susceptibility profile and resistance genes of *Salmonella* spp. on fish farms in the region of Grande Dourados / MS. The study was conducted in three fish farms where the first farm works with Lambari monoculture (*Astyanax lacustris*), the second with polyculture with four native species: Tambaqui (*Colossoma macropomum*), Pacu (*Piaractus mesopotamicus*), Dourado (*Salminus brasiliensis*) and Patinga (*Piaractus mesopotamicus*) and the third monoculture farm in Nile Tilapia (*Oreochromis niloticus*). Sampling occurred from October 2017 to May 2018. Eighty-four samples including twenty-four fish samples, forty-eight water and eighteen epilithic biofilms were analyzed. Thirteen *S. enterica* were isolated, identified by Chain Reaction of Polymerase and serotyped as *S.* Anatum, *S.* Minnesota, *S.* Panama and two isolates classified as *Salmonella enterica* subsp. *enterica*. The highest rates of resistance were to sulfonamides, trimethoprim, tetracycline and streptomycin. Multiple antibiotic resistance has been confirmed. Class 1 integrons have been detected. Genotypic characterization of *Salmonella* Panama was carried out and the bacterial genome was sequenced on an Illumina NextSeq®. *De novo* genome assembly was performed by CLC Genomic Workbench 12. The *Salmonella* Panama was assigned to ST2041. The aminoglycoside resistance gene, as well as genes encoding members of efflux pump and genes encoding resistance to biocides and heavy metals were found. In addition, the strain harbored genes that encode several virulence factors. Thus, the presence of *Salmonella* in the fish production chain demonstrates the wide distribution and adaptation of this microorganism. The antimicrobial resistance profile and genotypic characteristics call for caution regarding the use of antimicrobial agents to avoid the spread of resistance genes. The use of monitoring stations, such as epilitic biofilms, can be used as a sentinel in the detection of *Salmonella* in tanks on fish farms. It is known that the transmission of *Salmonella* spp. it usually occurs to man through the consumption of contaminated food, its mapping in the fish production chain provides subsidies for the development of measures to control epidemic outbreaks.

Keywords: Salmonellosis. Epilitic biofilms. Production animals.

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1 INTRODUÇÃO

A aquicultura é o setor da produção animal que mais vem crescendo nas últimas décadas por ser considerada uma promissora atividade econômica mundial, capaz de garantir emprego e renda aos profissionais envolvidos na área, além de fornecer alimento de elevado valor nutricional (SANTOS et al., 2019; DANTAS FILHO et al., 2020). Em 2018, a produção aquícola mundial atingiu outro recorde histórico de 114,5 milhões de toneladas (FAO, 2020). Tendência semelhante foi observada no Brasil que produziu 722.560 mil toneladas de peixe em 2019 com aumento de 4,5% de sua produção (PEIXE BR, 2020). Aliado ao crescimento da cadeia de produção, problemas sanitários relacionados à intensificação da atividade têm surgido (DANTAS FILHO et al., 2020). Patógenos alimentares como *Salmonella* têm sido envidenciados e a presença deste agente de doença transmitida por alimento é um grande problema de saúde pública em todo o mundo (ENG et al., 2015).

Salmonella spp. é um dos patógenos humano e animal mais importante (MICHAEL; SCHWARZ, 2016). Atualmente são reconhecidos aproximadamente 2.659 sorotipos de *Salmonella* e mais da metade deles pertencem à *Salmonella enterica* subsp. *enterica*, responsável pela maioria das infecções por *Salmonella* em humanos (ISSENHUTH-JEANJEAN et al., 2014; ENG et al., 2015). A transmissão de infecção por *Salmonella* não tifóide para os humanos pode ocorrer por meio da ingestão de alimentos ou água contaminados ou por contato direto com animais infectados (ENG et al., 2015). A cadeia produtiva animal é um dos principais reservatórios para muitos patógenos de origem alimentar e principal fonte de infecções por *Salmonella* (HEREDIA; GARCIA, 2018). Em seres humanos, diferentes sorotipos de *Salmonella* são capazes de causar gastroenterite, infecção que fica limitada ao intestino, mas que dependendo da imunidade do indivíduo e de fatores de virulência do microrganismo pode levar à morte. Os sinais e sintomas relacionados a salomonelose podem evoluir de náuseas, vômitos e diarreia para bacteremia e artrite reativa como uma sequela pós-infecção (EVANGELOPOULOU et al., 2015; SANTOS et al, 2019). Desta forma, a segurança microbiológica do pescado é uma preocupação dos consumidores, das indústrias e das agências reguladoras em todo mundo, uma vez que o pescado é um importante produto para comércio internacional de alimentos, sendo frequentemente exportado para vários países podendo ser veiculador de *Salmonella* em toda a cadeia produtiva (FERNANDES et al., 2018). Além da presença de *Salmonella,* outra preocupação recorrente é quanto ao uso de antibióticos na aquicultura. Na cadeia produtiva de peixe, assim

como em outras cadeias de produção animal, agentes antimicrobianos são utilizados para o controle de infecções bacterianas nos animais, porém o uso indevido ou inadequado dos antibióticos na aquicultura vem exercendo pressão seletiva o que resulta no aumento do isolamento de sorotipos de *Salmonella* resistentes e multidroga resistentes (CABELLO et al., 2013; ENG et al., 2015; FERNANDES et al., 2018).

Assim, verificar a presença de *Salmonella* spp. na cadeia produtiva de peixe em nossa região, identificar os pontos de um piscicultura em que *Salmonella* possa ser isolada, conhecer os principais sorotipos e o perfil de resistência são fundamentais para estabelecer estratégias de controle por meio de um manejo sanitário adequado.

2 REVISÃO DE LITERATURA

2.1 Aquicultura

Os recursos hídricos compõem a maior parte do planeta. Mudanças econômicas, sociais e demográficas em todo o mundo levaram ao crescimento da demanda de água para diversas atividades, dentre elas a aquicultura (LÓPEZ-DOVAL et al., 2017; GUAN et al., 2017). A aquicultura ou cultivo de organismos aquáticos é o segmento que mais cresce e que tem maior desenvolvimento dentre todas as cadeias de produção animal (ONO; KUBITZA, 1999; DIANA, 2009; DANTAS FILHO et al., 2020). Embora os detalhes do início da história e natureza da aquicultura não sejam claras, as pessoas têm cultivado peixes há milênios e há evidências de aquicultura em murais retratados em túmulos no Egito há 4000 anos, em livros escritos há 2300 anos na China e aquicultura costeira do Império Romano (LANDAU, 1992; COSTA-PIERCE, 2010). No entanto, a maior parte de seu crescimento e intensificação ocorreu nos últimos 30 anos, coincidindo com o rápido crescimento populacional do século XX, sendo que hoje a aquicultura é bem diferente dos sistemas históricos onde um crescimento expressivo mundial no setor tem apresentado um percentual de incremento médio de 3,2% ao ano no período 1961–2009 (FAO, 2012).

No Brasil, a produção e o consumo do pescado vêm crescendo a cada ano e o país possui potencial para o desenvolvimento sustentável de pisciculturas, pois é constituído por 5,5 milhões de hectares de reservatórios de águas doces, clima favorável e disponibilidade de mão-de-obra (BRASIL, 2006). Dados apontam que em 2019 o Brasil produziu 722.560 mil toneladas de peixe, com incremento de 4,5% de produção. O Relatório da Organização das

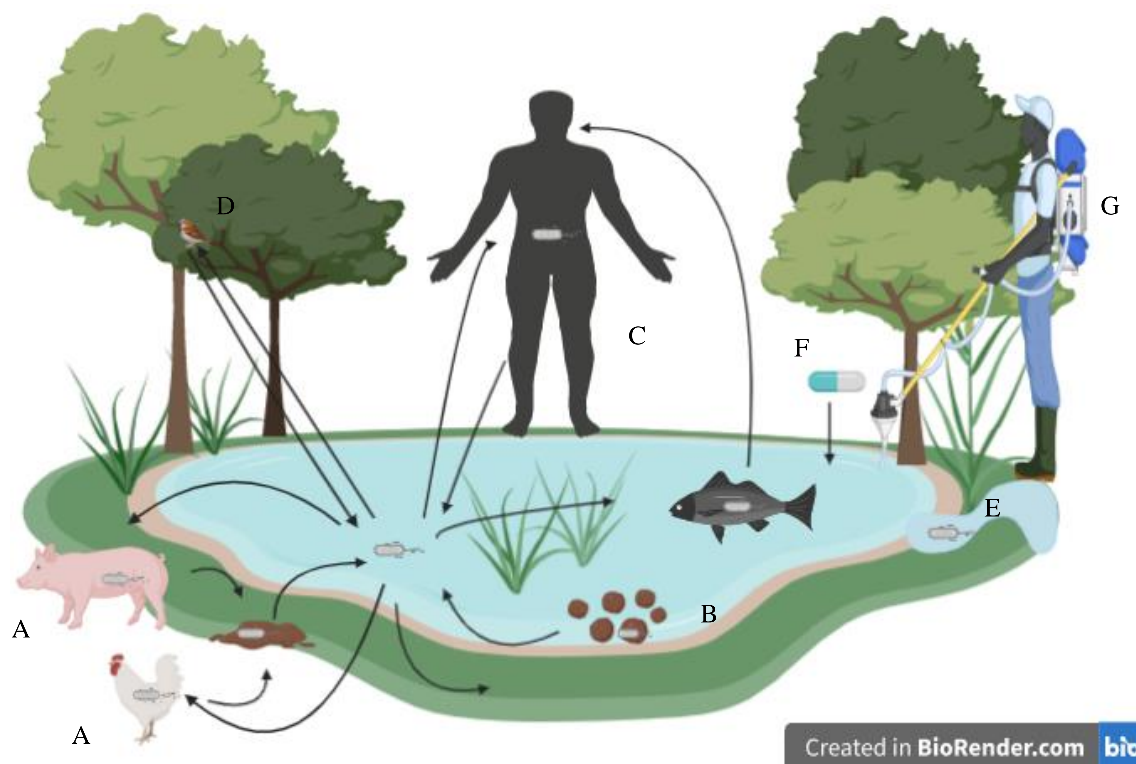
Nações Unidas para a Alimentação e a Agricultura (FAO) estima que o País registre crescimento de 104% na pesca e aquicultura até 2025 (FAO, 2016; PEIXE BR, 2020).

Todo este aumento pode ser atribuído ao fato da aquicultura ser considerada pela ONU como uma atividade estratégica à segurança alimentar mundial, por disponibilizar mais rapidamente fontes de proteína para a população quando comparado com outros animais de criação, gerar emprego, riqueza e outro fator é o aumento da busca da carne de pescado pelos consumidores brasileiros, como alternativa saudável, enquanto alimento proteico (SILVA; LOSEKANNH; HISANO, 2013; DANTAS FILHO et al., 2020). A carne de peixe é considerada um alimento rico em nutrientes, proteínas, ácidos graxos, vitaminas e minerais, e seu consumo é classificado como alimento funcional, capaz de reduzir os riscos de doenças coronarianas, acidente vascular cerebral, degeneração macular relacionada à idade, promover o desenvolvimento normal do cérebro e contribuir para uma expectativa global de vida mais longa (KRIS-ETHERTON; HARRIS; APPEL, 2002; VILA NOVA; GODOY; ALDRIGUE, 2005; BENÍTEZ SANTANA et al., 2007; RAMOS FILHO et al., 2008; COSTA, 2007; TACÓN; METIAN, 2013).

Apesar do crescimento e das animadoras perspectivas do setor, são grandes os desafios diante de alguns obstáculos dentro da produção de pescado. O impressionante desenvolvimento tem sido acompanhado de algumas práticas potencialmente danosas para o meio ambiente, saúde animal e humana, visto que a produção de peixes depende da entrada de alimentos formulados (rações que contenham antibióticos, ingredientes proteicos de origem animal e vegetal, tais como farinhas de sangue, penas hidrolisadas, carne, carne e ossos, vísceras de frango, resíduo de pescado e coprodutos ou resíduos agroindustriais), da aplicação de agroquímicos, antibióticos, fertilização com excretas animais (utilização de cama de frango para fertilização de tanques), criação associada com outros animais (criação conjunta com gado, frangos, suínos, cavalos e outros animais), escoamento de resíduos (produtos agroquímicos utilizados em lavouras ao redor), o que leva à presença de muitos contaminantes químicos e biológicos para o produto final e ao meio ambiente (Figura 1) (SAPKOTA et al., 2008). Os contaminantes microbiológicos podem comprometer a cadeia produtiva do pescado, tornar-se um perigo à saúde pública, gerar prejuízos econômicos e problemas sanitários. Atualmente, um dos desafios para o desenvolvimento da cadeia produtiva do pescado é a presença de *Salmonella* (LEAL et al., 2017; FERNANDES et al., 2018; DANTAS FILHO et al., 2020). A presença de *Salmonella* em aquicultura vem sendo apontada como um dos maiores e mais impactantes desafios sanitários devido à dificuldade no

seu controle considerando as características intrínsecas da atividade como do ambiente aquático do qual possui condições favoráveis como presença de matéria orgânica, umidade e temperatura, que favorecem a proliferação bacteriana e sua transmissão (SANTOS et al. 2019; FERNANDES et al., 2018; DANTAS FILHO et al., 2020; LEAL; OLIVEIRA; FIGUEIREDO, 2017). Assim, a presença e multiplicação de microrganismos patogênicos, como a *Salmonella* spp., em produtos alimentares são uns dos principais alvos dos sistemas de controle e monitoramento de microrganismos no abate de animais no Brasil e no mundo devido à sua importância como doença transmitida por alimento (DTA) (SANTOS et al., 2019, DANTAS FILHO et al., 2020).

Figura 1 - Ciclo caracterizando as possíveis rotas de *Salmonella* em pisciculturas através de A) criação consorciada com outros animais, B) utilização de ração para peixe com proteína animal, C) homem, D) animais silvestres, E) água de entrada dos tanques e também entrada de agentes antimicrobianos como F) antibióticos e G) biocidas em pisciculturas.



Fonte: Autor (2020). Criado com BioRender.com.

2.2 *Salmonella*

Salmonella é uma bactéria Gram-negativa, anaeróbica facultativa, em forma de bastonete curto de 0,7-1,5 x 2-5 µm, não esporogênica, não encapsulada, intracelular facultativa, podendo sobreviver no interior de fagócitos e se multiplicar intracelularmente (HANSEN-WESTER; HENSEL, 2001; POPOFF; LE MINOR, 2005; WANG et al., 2015). São bactérias mesófilas, apresentam crescimento em temperaturas variando de 5 a 45 °C, com temperatura de crescimento ótimo entre 35 e 37 ºC. Possuem uma relativa resistência ao calor e substâncias químicas, porém não sobrevivem à temperatura de 55 ºC em 1 h ou em 60 ºC de 15 a 20 minutos, enquanto que o processo de congelamento leva apenas a uma redução significativa do número de células viáveis, não sendo capaz de provocar a destruição completa (HOLT, 1994; GAST, 1997; WALTMAN, 2000; D'AOUST; MAURER, 2007; JARVIS et al., 2016).

Em relação ao pH, elas crescem no intervalo de 4,5 a 9,0, com crescimento ótimo na faixa de 6,5 a 7,5, (pH da maioria dos alimentos de origem animal). Geralmente em pH abaixo de 4,0 e acima de 9,0 são destruídas lentamente. As salmonelas são quimioheterotróficas e têm a capacidade de metabolizar nutrientes tanto pela via respiratória quanto pela via fermentativa (D'AOUST; MAURER, 2007). A maioria dos sorotipos produz gás a partir da fermentação da glicose, com exceção de *S*. Typhi, *S*. Pullorum e *S*. Gallinarum, e também fermentam arabinose, maltose, manitol, manose, ramnose, sorbitol, trealose, xilose e dulcitol, produzem gás sulfídrico (H_2S) a partir da redução do enxofre por ação da enzima cisteína desulfidrase, podendo utilizar também o citrato como fonte de carbono, catalase, lisina e ornitina descarboxilase positiva. São oxidase positivas, Voges-Proskauer (VP), Vermelho de Metila (VM), malonato, urease e indol negativos, e reduzem o nitrato a nitrito (JONES; McLAREN; WRAY 2000; GAST, 2008; BRASIL, 2011) (Tabela 1).

Tabela 1 - Características bioquímicas de *Salmonella* spp.

Meios	Reação
Ureia	Reação negativa
LDC – Lisina descarboxilase	Positivo: Reação alcalina (cor púrpura do meio) e amarelo no meio controle (exceto *S*. Paratyphi)

ODC – Ornitina descarboxilase	Positivo: Reação alcalina (cor púrpura do meio) e amarelo no meio controle (exceto *S*. Gallinarum – ODC negativo)
VM – Vermelho de metila	Coloração vermelha do meio após adição do reagente
VP – VogesProskauer	Meio sem alteração após adição dos reagentes
Fenilalanina desaminase	Meio inalterado após colocação do reagente
Utilização do citrato	Positivo: Reação alcalina – cor azul do meio
Indol	Negativo – anel amarelo após colocação do reagente – Kovacs
H_2S	Presença de pigmento negro de qualquer intensidade ou somente na picada (cepas imóveis)
Motilidade	Crescimento ao longo da picada (imóvel) ou turvação do meio (móvel)
Glicose	Positivo (reação ácida) com/sem gás no tubo de Durhan
Lactose	Negativo/Positivo
Manitol	Positivo
Sacarose	Negativo

Fonte: Adaptado de Brasil (2011).

Suas características bioquímicas podem sofrer variações em função do sorotipo e/ou da subespécie (BRASIL, 2011) e também devido a mutações e trocas conjungativas de plasmídeos intra e inter-gênero. A maioria das salmonelas de interesse clínico não fermenta

lactose, contudo diversos estudos já relataram a ocorrência de estirpes de *Salmonella* spp. portadores de plasmídeos que codificam enzimas que permitem a fermentação de lactose (isolados lac+) e de sacarose (isolados sac+) (KUMAR; SURENDRAN; THAMPURAN, 2009; BRASIL, 2011).

As salmonelas apresentam estruturas morfológicas como: flagelos, lipopolissacarídeos (LPS), fímbrias, e algumas proteínas da membrana externa que atuam na adesão e/ou invasão do epitélio do trato intestinal. A maioria possui numerosas fímbrias para aumentar a capacidade de fixação a vários substratos. Exceto os sorotipos Pullorum e Gallinarum, que são isentos de flagelos sendo assim imóveis, a maioria é móvel pela presença de flagelos peritríquios (HOLT, 1994; WILSON et al., 2000; BERNDT et al., 2007; GERMANO; GERMANO, 2008).

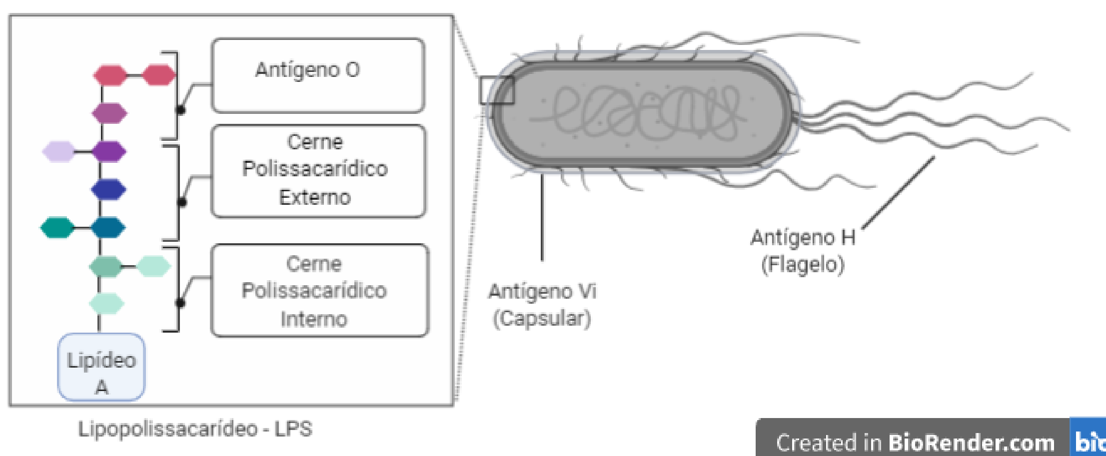
Salmonella apresenta três antígenos principais: antígeno O (ou antígeno somático), antígeno H (ou antígeno flagelar) e antígeno Vi (ou antígeno capsular) (Figura 2). Os antígenos O (Ohne), antígenos somáticos, são polissacarídeos termoestáveis localizados na porção mais externa da parede celular bacteriana. São polímeros de subunidades O compostos por 4 ou 6 açúcares. Essas subunidades formam regiões altamente variáveis onde os tipos de arranjos dos açúcares, determinam diferentes epítopos e assim, a especificidade de cada sorotipo. Em sua estrutura podem ser distinguidas duas classes de epítopos específicos: os antígenos do grupo O, associados com a configuração do carboidrato central da estrutura do antígeno O; e os antígenos ancilares O, que são carboidratos adicionais à estrutura central do antígeno O. Foram descritos 67 antígenos O e seus epítopos permitem a classificação das salmonelas em 46 sorogrupos distintos (KONEMAN et al., 2001; PARIJA, 2009; CDC, 2011).

O antígeno H (Hauch), antígeno flagelar, é uma porção filamentosa do flagelo bacteriano, constituído de subunidades proteicas denominadas flagelinas (H), que são altamente imunogênicas. Tal fato está associado com a formação de anticorpos logo após a infecção ou imunização, instáveis na presença de calor (termolábeis) e de sensibilidade a álcool e ácidos. As porções N e C terminais da flagelina, são conservadas e conferem a estrutura filamentosa característica. A região antigenicamente variável da flagelina é a porção mediana a qual fica exposta na superfície do flagelo (RYCROFT, 2000; GRIMONT; WEILL, 2007; CDC, 2011). Este antígeno pode estar presente na forma simples (monofásica) ou em duas formas separadas (difásica). Contudo, somente uma delas é expressa a um dado momento. Esse fenômeno é conhecido como variação de fase de Andrewes. Isso ocorre devido à redução ou perda da expressão dos genes para diferentes tipos de flagelina que,

ajudam na sobrevivência da bactéria frente às defesas do hospedeiro ou de forma natural, como ocorre em *S*. Enteritidis e *S*. Typhi. Todavia, a maioria dos sorotipos apresentam tanto os antígenos H1 quanto os antígenos H2, sendo assim denominados difásicos. Para a identificação completa de um sorotipo é necessária a detecção do antígeno H em ambas as fases (MACNAB, 1987; GRIMONT; WEILL, 2007; PARIJA, 2009; CDC, 2011).

Um terceiro antígeno é o antígeno capsular Vi (Félix e Pitt), composto basicamente por polissacarídeo, sobrejacente ao antígeno O, instável na presença de calor (termolábil) e sensível a ácido fenol hidroclórico e hidróxido de sódio 0,5%. É nomeado de Vi devido à sua associação com a virulência, pois cepas que o possuem são mais virulentas quando comparadas às cepas que não o possuem. Três locos gênicos estão envolvidos no controle genético de sua produção (*viaA, viaB and ompB*) e somente os sorotipos Typhi, Paratyphi C e Dublin expressam essa característica. Aqueles que o possuem, têm seus antígenos O encobertos e se tornam incapazes de aglutinar com o anti-soro O (GRIMONT; GRIMONT; BOUVET 2000; RYCROFT, 2000; PARIJA, 2009).

Figura 2 - Representação dos antígenos O, H e Vi, compreendendo o esquema de sorotipagem definido por Kauffman-White-Le Minor.



Fonte: Adaptado de CHENG et al, 2019. Criado com BioRender.com.

2.3 Histórico

O primeiro relato de uma bactéria considerada *Salmonella* se deu em 1880 por Karl Joseph Eberth. Médico e estudante de Rudolf Virchow, Eberth isolou e observou pela primeira vez um bacilo tifoide em baço e linfonodos mesentéricos de humanos e designou como *Bacterium typhosa,* responsável pela febre tifoide. Quatro anos depois o bacilo foi

isolado em meio de cultura pelo bacteriologista George Theodor August Gaffky (CORRÊA; CORRÊA, 1992; MARINELI et al., 2013). Posteriomente em 1885, o microbiologista veterinário do Departamento de Agricultura dos EUA, Daniel Elmer Salmon e o epidemiologista e patologista Theobald Smith isolaram em suínos outro membro do gênero *Salmonella* o qual foi erroneamente denominado de *Bacillus cholerae suis* (BRASIL, 2011; MARINELI et al., 2013). Em 1888 na Alemanha, August Anton Hieronymus Gärtner isolou pela primeira vez em laboratório, *Bacterium enteritidis* (posteriormente denominada de *Salmonella enteritidis*) da carne bovina e dos órgãos de um homem que apresentava quadro de gastroenterite durante um surto de intoxicação na cidade de Frankenhausen localizada no distrito de Kyffhauser (GÄRTNER apud ANDREWS; BAUMLER, 2005, 1888; BARROW, 1993). Em 1900, o cientista francês Joseph Léon Ligniéres sugere uma mudança de nomenclatura, nomeando o gênero do bacilo de "*Salmonella*". Este nome foi dado como homenagem ao cientista americano Daniel Salmon (BARROW, 1993; POPOFF; LE MINOR, 2005; BRASIL, 2011; MARINELI et al., 2013).

Desde então, várias alterações na nomenclatura do gênero *Salmonella* foram propostas. No início do século XX, a caracterização das bactérias do gênero *Salmonella* ainda era confusa. A partir de 1920, começou a ficar mais clara quando um grupo de microbiologistas, liderados por Fritz Kauffmann, em Copenhagen, e por Philip Bruce White, em Londres, unificaram a taxonomia. Kauffmann e White desenvolveram um esquema de sorotipagem, designando um sistema de classificação para *Salmonella* spp. que em 1933 foi reconhecido pelo subcomitê de *Salmonella* da Sociedade Internacional de Microbiologia, como esquema de Kauffmann-White (POPOFF, LE MINOR, 2005; MARINELI et al., 2013; BRASIL, 2011).

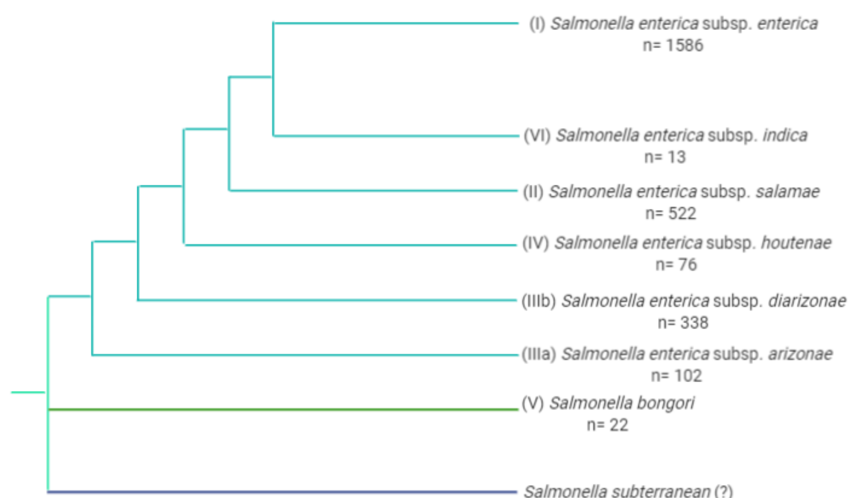
Em sua primeira publicação, em 1934, o esquema de Kauffman-White listou 44 sorotipos de *Salmonella* spp. Trinta anos depois, em 1964, com auxílio de Leon Le Minor, pesquisador do Instituto Pasteur, o esquema possuía 958 sorotipos e, atualmente conta com 2.659 sorotipos descritos. Devido à grande contribuição de Le Minor na publicação da maioria dos sorotipos conhecidos hoje, o esquema antes denominado somente "Kauffmann-White" passou também a ser conhecido como "esquema de Kauffmann-White-Le Minor", sendo esta nomenclatura menos difundida (SALMONELLA SUBCOMMITTEE, 1934; ISSENHUTH-JEANJEAN et al., 2014). A partir de então, a nomenclatura passou por algumas modificações, tendo por base a utilização de métodos clássicos e de métodos moleculares, como AFLP (Amplified Fragment Length Polymorphism /Amplified Fragment

Length Polymorphism), sequenciamento de rRNA 16S e MLEE (eletroforese de enzimas multilocus/ multilocus enzyme electrophoresis) (BRASIL, 2011).

2.4 Taxonomia e Nomenclatura

Salmonella é uma bactéria pertencente ao filo Proteobacteria, classe Gammaproteobacteria, ordem Enterobacteriales, família Enterobacteriaceae e gênero *Salmonella* (POPOFF; LE MINOR, 2005). Uma nomenclatura que reflete os recentes avanços na taxonomia do gênero de *Salmonella* recomendada pela Organização Mundial de Saúde (OMS), através do Centro de Referência e Pesquisa, classifica o gênero *Salmonella* em duas espécies: *S. bongori* e *S. enterica,* as quais possuem atualmente cerca de 2659 sorotipos, cuja distribuição editada por Issenhuth-Jeanjean et al., 2014 é apresentada na Figura 3 (POPOFF, LE MINOR, 1997; SU; CHIU, 2007; MILLER; WIEDMANN, 2016). Uma terceira espécie denominada de *Salmonella subterranean* foi prosposta por Shelobolina et al. em 2004, porém, ainda não é aceita pela comunidade científica, pois segundo Grimont & Weill (2007) e Issenhuth- Jeanjean et al., (2014), esta espécie não pertence ao gênero *Salmonella*.

Figura 3 - Árvore filogenética com 2659 sorotipos distribuídos em cada espécie e subespécie de *Salmonella.*



Fonte: Adaptado de CHENG et al, 2019; ISSENHUTH-JEANJEAN et al., 2014, Shelobolina et al., 2004. Criado com BioRender.com.

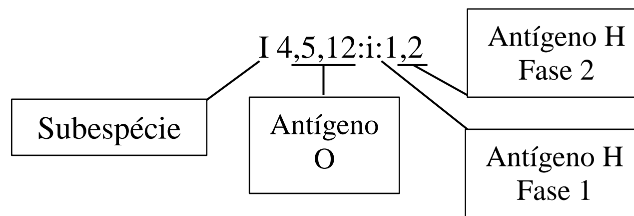
Com base em reações bioquímicas, por meio da utilização de açúcares, proteínas e a produção de gases, a espécie *S. enterica* foi subdividida em seis subespécies, designadas por nomes ou por números romanos: *enterica(I), salamae(II), arizonae(IIIa), diarizonae(IIIb), houtenae(IV)* e *indica(VI)* (BRENNER et al., 2000; POPOFF et al., 2001; TINDALL et al., 2005; GRIMONT; WEILL, 2007; FOLEY; LYNNE, 2008; DUNKLEY et al., 2009, ISSENHUTH-JEANJEAN et al., 2014).

Entre as duas espécies, a *Salmonella enterica* subsp. *enterica* apresenta maior número de sorotipos, representando 99% dos isolamentos. Esses sorotipos são tipicamente designados por um nome geralmente relacionado ao local onde o sorotipo foi isolado pela primeira vez e na forma da redação da nomenclatura. Os sorotipos não são mais considerados espécies, razão pela qual os sorotipos da subespécie enterica devem ser designados da seguinte maneira: *Salmonella typhimurium*, *Salmonella enterica* subsp. *enterica* sorotipo Typhimurium ou, de maneira mais simples e objetiva, *Salmonella* sorotipo Typhimurium ou ainda *Salmonella* Typhimurium. Portanto, o nome do sorotipo é escrito em letras romanas não itálicas e a primeira letra maiúscula (GRIMONT; WEILL, 2007; BRASIL, 2011; ISSENHUTH-JEANJEAN et al., 2014).

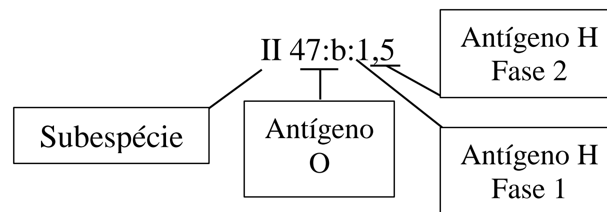
Os sorotipos são designadas por suas fórmulas antigênicas, seguindo o esquema de Kauffmanne-Whitee-Le Menor onde a classificação é determinada pela combinação de seus antígenos O, H1 (flagelar fase1) e H2 (flagelar fase2). Com base nessas características, a sorotipagem é descrita da seguinte forma: para os antígenos O são atribuídos algarismos arábicos (Ex.: 1, 2 e 5) enquanto que, para os antígenos H1 são atribuídos letras minúsculas (Ex.: a, b, i) e para antígenos H2 numerais arábicos (Ex.: 1, 2 e 5) (SELANDER; LI; NELSON, 1996; JAY, 2000; GRIMONT; WEILL, 2007; ISSENHUTH-JEANJEAN et al., 2014).

Abaixo seguem dois exemplos da forma de descrição da fórmula antigênica, onde a subespécie é representada por algarismo romano, espaço, antígeno O (representado por algarismo arábico) e letras do alfabeto latino, dois pontos, Antígeno H de fase 1 representado por algarismo arábico, dois pontos, Antígeno H de fase 2 representado por algarismo arábico:

A) Fórmula antigênica correspondente a *Salmonella enterica* subespécie *enterica* sorotipo Typhimurium.



B) Fórmula antigênica correspondente a *Salmonella enterica* subespécie *salamae* sorotipo 47:b:1,5



2.5 Habitat

As salmonelas são bastante difundidas geograficamente em todo o mundo, residem como flora normal no trato gastrointestinal de seus hospedeiros, porém seus reservatórios são diversos, pois sua constituição genética permite sua adaptação a uma variedade de ambientes e animais, e dependendo do sorotipo, incluem animais de sangue quente e frio, mas também plantas, solo e água (CARRASCO; MORALES-RUEDA; GARCIA-GIMENO, 2012; KE et al., 2014; ANDINO; HANNING, 2015).

A maioria das subespécies de *Salmonella enterica* e de *Salmonella bongori* são comensais de animais de sangue frio e estão associadas ao meio ambiente (BRENNER et al., 2000). *Salmonella enterica* subsp. *enterica* está principalmente associada a infecções em animais de sangue quente, sendo conhecida como um patógeno zoonótico (ISSENHUTH-JEANJEAN et al., 2014; KE et al., 2014). Compreende a maioria dos sorotipos patogênicos, especialmente os não-tifoides, responsáveis pela salmonelose alimentar, sendo este um dos patógenos alimentares mais importantes, levando a milhões de casos de doenças entéricas, milhares de hospitalizações e mortes em todo o mundo a cada ano (MEAD et al., 1999; GALANIS et al., 2006; CDC, 2009; DUNKLEY et al., 2009; HENDRIKSEN et al., 2009; CALLAWAY et al., 2010; PUAH; CHUA; TAN, 2017).

Esta bactéria tem sido isolada de diferentes animais de produção como: aves, ovinos, suínos, peixes e frutos do mar (HEREDIA; GARCÍA, 2018). No Brasil um levantamento epidemiológico realizado no período de 2000 a 2009 apontou um panorama global dos

sorotipos de *Salmonella* spp. circulantes no país, retratando a incidência de *Salmonella* spp. em diferentes fontes da cadeia epidemiológica, incluindo isolados de fonte humana, alimentar, animal, ambiental e rações (RODRIGUES et al., 2010).

Trabalhos evidenciaram a importância de *Salmonella* na cadeia produtiva do pescado (FERNANDES et al., 2018; DANTAS FILHO et al., 2020; LEAL et al., 2017). A contaminação desses alimentos pode ocorrer na própria fonte de produção, isto é, a partir dos animais criados nas fazendas que podem ser os portadores do agente (DANTAS FILHO et al., 2020). Alguns fatores no manejo em tanques de peixe como a utilização de estercos, como a criação consorciada com animais e uso de ração com fonte proteíca de origem animal. Estas condições podem propiciar a contaminação de *Salmonella* na cadeia produtiva, os quais contribuem para o aumento da população de salmonela nos tanques (FERNANDES et al., 2018).

2.6 Salmonelose

A salmonelose é um importante problema de saúde pública, sendo uma das principais causas de morbidade em todo o mundo. A maioria das infecções está associada à ingestão de alimentos contaminados, contato e consumo de água não potável e também com contato direto com animais infectados (BENENSON; CHIN, 1995; GOMEZ et al., 1997; BRANDS et al., 2005; MEDUS et al., 2006; ZHAO et al., 2008; ENG et al., 2015). Diferentes sorotipos são responsáveis por infecções clínicas, subclínicas a graves em animais de criação, animais de estimação, bem como febre tifoide e gastroenterite em humanos (EVANGELOPOULOU et al., 2015). Estas bactérias são os principais agentes etiológicos de gastroinfecções relacionada com alimentos em humanos (KIRK et al., 2015).

No Brasil, a *Salmonell*a está em segundo lugar entre os 10 agentes etiológicos mais identificados nos surtos de Doenças transmitidas por alimento em dados de 2009 a 2018 (MINISTERIO DA SAÚDE, 2019). Surtos infecciosos de origem alimentar têm sido registrados há vários anos e, no que se refere ao pescado, recentemente, o número de relatos de casos de peixes para consumo infectados por *Salmonella* aumentou devido à maior demanda de pescado, principalmente na Europa, Estados Unidos, Oeste da África, Oriente Médio e no Brasil (DANTAS FILHO et al., 2020). Assim sendo a investigação deste agente na cadeia produtiva do pescado um importate método para melhoria da qualidade do produto para o consumidor.

2.7 Resistência aos antimicrobianos

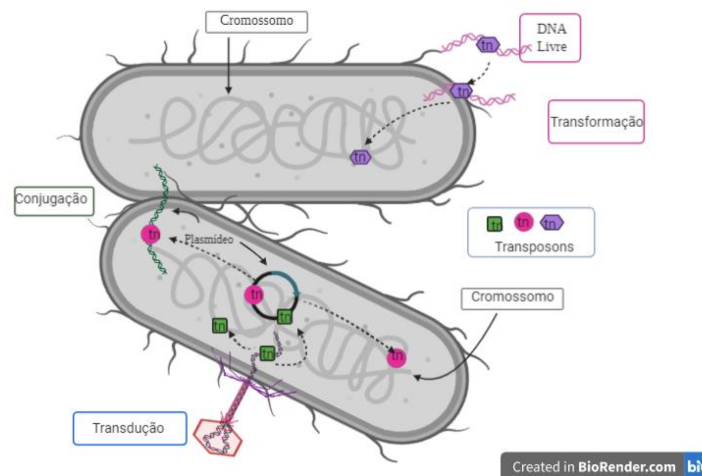
A resistência antimicrobiana é definida como a capacidade dos microrganismos de resistir aos efeitos dos agentes antimicrobianos (SANSEVERINO et al., 2018). Bactérias podem adquirir genes de resistência em uma variedade de cenários envolvendo seres humanos, animais e o meio ambiente. As fazendas e indústrias alimentícias dependem, em grande parte, do uso de antibióticos, metais pesados e biocidas, e outros agentes antimicrobianos e conservantes com propriedades antimicrobianas, a fim de fornecer alimentos de alta qualidade microbiológica e seguros para os consumidores. No entanto o uso intensivo de agentes antimicrobianos causa uma pressão seletiva, criando reservatórios de bactérias resistentes aos antimicrobianos sendo este o principal fator do surgimento de isolados de microrganismos resistentes. Além disso, possibilita a transferência horizontal e a recombinação genética de genes de resistência antimicrobiana entre bactérias de diferentes esferas ecológicas (SANDAA; ENGER, 1994; KRUSE; SORUM, 1994; RHODES et al., 2000; L'ABEE-LUND; SØRUM, 2001; BARBER; MILLER; MCNAMARA, 2003; SØRUM, 2006; SHAH et al., 2014; MONTE et al., 2019).

Além do mais, bactérias expostas a compostos, como biocidas e metais pesados, podem promover a resistência aos antibióticos por meio de um fenômeno denominado co-seleção. A co-seleção ocorre quando um mecanismo bioquímico induz resistência a diferentes compostos (resistência cruzada), quando os genes que conferem fenótipos resistentes estão localizados no mesmo elemento genético (co-resistência), ou quando um gene de resistência para diferentes substâncias é regulado por um único gene regulador (co-regulação) (SANSEVERINO et al., 2018).

As bactérias podem transferir seu conteúdo genético intra e inter-gênero. Genes de resistência antimicrobiana são carregados em elementos genéticos móveis, como plasmídeos, trasposons e integrons, que podem se disseminar por transferência horizontal ou vertical (AMAGLIANI; BRANDI; SCHIAVANO, 2012). A transferência vertical de genes permite que a progênie subsequente mantenha o fenótipo de resistência. Porém o maior problema de saúde pública é a transferência horizontal de genes, que pode ocorrer através de 3 mecanismos: conjugação entre bactérias patogênicas e comensais (célula a célula), por transformação (captação de fragmentos de DNA) ou por transdução (por fago) durante a qual o fago é responsável pela inserção de genes de resistência (Figura 4) (THOMAS; NIELSEN, 2005; MONTE et al., 2019). A transferência horizontal pode ocorrer entre bactérias de diferentes hospedeiros incluindo animais terrestres, peixes e humanos e por meio de várias

rotas. Assim a transferência de genes de resistência para animais que são utilizados como alimento ao homem exerce um importate papel na transmissão de microrganismos resistentes de animais para humanos (AMAGLIANI; BRANDI; SCHIAVANO, 2012).

Figura 4 - Mecanismos de transferência genética em bactérias.



Fonte: autor (2020). Criado com BioRender.com.

2.8 Mecanismo de disseminação de resistência mediado por integrons

A resistência aos antibióticos é principalmente transferida entre as bactérias por elementos genéticos móveis (EGM). Fundamentalmente, os integrons não são categorizados como elementos genéticos móveis, mas uma estreita associação entre integrons e elementos genéticos móveis, como transposons, sequências de inserção e plasmídeos conjugativos, facilitam suas frequentes transmissões intraespécies e interespécies (KAUSHIK et al., 2018,2019).

Os integrons são sistemas de expressão gênicos bem organizados que promovem a captura de um ou mais conjuntos de genes não funcionais e os converte em genes expressos com o mesmo sítio de ligação, formando assim clusters compostos de genes de resistência a antibióticos (HALLA; COLLIS, 1995; RECCHIA; HALL, 1995; CARATTOLI, 2001; SU et al., 2012). São compostos de três componentes : (1) o gene *integrase* (*intI*1), que codifica uma enzima tirosina recombinase específica do local, integrasse, responsável pela integração e excisão de genes cassetes; (2)(*attI)* o sítio de ligação e (3) um promotor (Pc) incorporado no gene *intl*, orientado para o ponto de integração, que regula a expressão de conjuntos cassetes de genes (KAUSHIK et al., 2018; DENG et al., 2015).

Os integrons podem espalhar facilmente a resistência aos antibióticos e contribuem para a disseminação e associação com a multirresistência aos antimicrobianos em membros da família Enterobacteriaceae (LEVERSTEIN-VAN HALL et al., 2002). Os integrons têm sido relatados frequentemente em cepas multirresistentes isoladas de animais e humanos, e são localizados no cromossomo bacteriano ou em plasmídeos de uma ampla gama de hospedeiros (CARATTOLI, 2001). Existem 5 tipos de integrons, integrons de classe 1, 2, 3, 4 e 5 cuja classificação é de acordo com o gene da integrase (*intI*). Entretanto apenas as três primeiras classes estão associadas à dispersão de múltiplos genes de resistência em Enterobacteriaceae (KAUSHIK et al., 2018).

A classe 1 é a mais abundante em bactérias entéricas, incluindo todos os patógenos comuns como *Salmonella* (KAUSHIK et al., 2018). Sendo essa classe a mais prevalente do que outras classes de integrons devido à sua capacidade de recombinar com locais *att*C díspares e até mesmo com sequências nucleotídicas com menor semelhança. O integron de classe 1 está associado ao transposon Tn402, que é frequentemente inserido dentro de um transposon grande, como Tn21 / Tn1696 , e carrega determinantes de resistência a antibióticos dentro das duas sequências conservadas (5’CS e 3’CS). O 3’CS inclui: *qacEΔ1*, um gene de resistência a detergente truncado; um gene *sul1* para resistência a sulfonamida; e orf5, um quadro de leitura aberto com função desconhecida (DENG et al., 2015). Mais de 70 cassetes de genes diferentes que conferem resistência para a maioria dos β-lactâmicos conhecidos, aminoglicosídeos, trimetoprim, compostos de rifampicina, cloranfenicol, quinolonas, eritromicina e quaternário de amônio são relatados nesta classe de integron (KAUSHIK et al., 2018). Ao contrário dos integrons da classe 1, os integrons da classe 2 são menos prevalentes nas bactérias entéricas. Os integrons da classe 2 são geralmente associados a genes que conferem resistência ao trimetoprim (*dfrA1*), estreptotricina (*sat2*) e estreptomicina (*aadA1*) (DENG et al., 2015).

2.9 Mecanismos de disseminação de Resistência mediada por plasmídeo

Os plasmídeos são em sua maioria replicons independentes de fita dupla de DNA circular extracromossômico. Eles possuem uma variedade de tamanhos desde pequenos, com menos de uma dezena de kilobase, a muitas vezes crípticos a grandes, megaplasmídeos com algumas centenas de kilobases com muitos recursos que permitem que eles se adaptem a diferentes condições ambientais (PIOTROWSKA; POPOWSKA, 2015). A grande maioria dos plasmídeos carrega uma série de determinantes diferentes como genes de resistência a

antibióticos, a metais ou fatores de virulência que conferem diversas propriedades à bactéria (QUINN et al., 2011; PIOTROWSKA; POPOWSKA, 2015).

Os plasmídeos movem genes promiscuamente entre espécies e gêneros bacterianos (HALL; BARLOW, 2004). Em *Salmonella,* os plasmídeos desempenham um papel importante na transmissão e disseminação de determinantes de resistência antimicrobiana entre as cepas e podem ser transmitidos por meio do processo conhecido como conjugação (JAJERE, 2019). Plasmídeos híbridos portadores de genes de resistência antimicrobiana e de fatores de virulência foram descritos em vários sorotipos de *Salmonella* (GUERRA et al., 2002). Plasmídeos com multirresistência contendo integrons de classe 1 ou plasmídeos híbridos de resistência e virulência podem desempenhar um papel importante na co-seleção, manutenção e disseminação da resistência antimicrobiana entre isolados de *Salmonella* em sistemas de produção animal (LOPES et al., 2016).

Vários estudos têm reproduzido experimentalmente a transferência de genes de resistência entre sorotipos de *Salmonella* e outras espécies bacterianas via plasmídeos conjugativos (GUERRA et al., 2002; LOPES et al., 2016). Algumas famílias de plasmídeos (grupo de incompatibilidade Q, P, F, L, HI, N, L/M) são altamente prevalentes em enterobactérias emergindo em conjunto com genes de resistência clinicamente importantes (CARATTOLI, A. , 2003; MOREIRA et al, 2013).

2.10 Produção animal e Resistência antimicrobiana de *Salmonella*

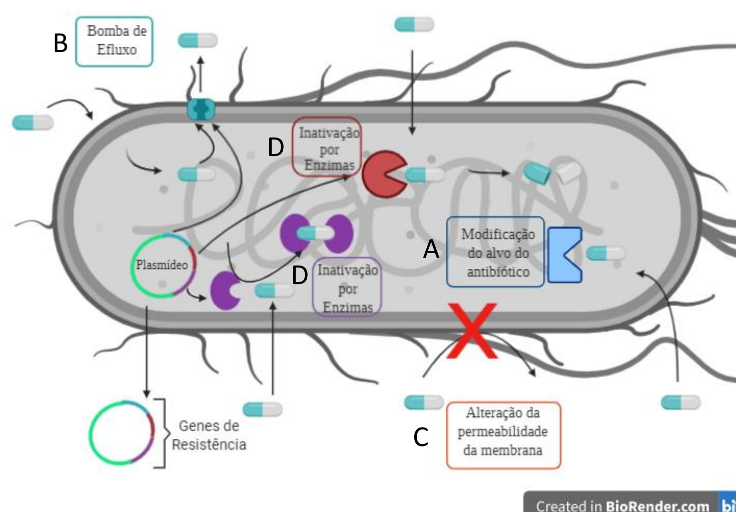
Animais de produção são reservatórios de patógenos com potencial para transferir resistência a antimicrobianos para humanos (WHO, 2017). A cadeia de produção de alimentos é um ecossistema altamente diversificado, povoado por comensais e bactérias patogênicas como *Salmonella,* da qual é uma das espécies incluídas nas listas de bactérias dos programas de vigilância e monitoramento da Organização das Nações Unidas para a Agricultura e a Alimentação (FAO), Organização Mundial da Saúde (OMS), Organização Mundial para Saúde Animal (OIE) e Codex Alimentarius que têm investigado resistência antimicrobiana em animais, humanos e alimentos (WHO, 2017).

Bactérias patogênicas como *Salmonella,* têm sido motivo de preocupação na cadeia produtiva de alimentos de origem animal. Cepas multirresistentes de *Salmonella* estão frequentemente sujeitas a rearranjo genético, devido à dinâmica da bactéria frente à

diversidade de hospedeiros e variações do ambiente no qual se encontram (MOREIRA et al., 2013). A emergência de *Salmonella* resistente a antimicrobianos é um sério problema mundial de saúde pública (CHIU et al., 2002). No início dos anos 60 foi relatada pela primeira vez resistência de *Salmonella* para apenas cloranfenicol (ENG et a., 2015). Desde então tem aumentado a incidência de isolados de *Salmonella* apresentado resistência para um ou mais agentes antimicrobianos e uma ampla gama de surtos causados por *Salmonella* multirresistente tem sido relatada em todo o mundo (LAMAS et al.,2018). A resistência a diversos antibióticos apresentada por isolados de *Salmonella* spp. está associada, principalmente, com as classes mais antigas de antibióticos, como cloranfenicol, trimetoprim-sulfametoxazol e alguns β-lactâmicos como ampicilina e cefalosporinas de 1ª geração (HUR et al., 2012; ANTUNES et al., 2016). Agentes antimicrobianos como ampicilina, cloranfenicol e trimetoprim-sulfametoxazol são usados como tratamentos tradicionais de primeira linha para infecções por *Salmonella* (ENG et al., 2015). Assim, com o surgimento de resistência aos antibióticos tradicionais, outros antibióticos como fluoroquinolonas e cefalosporinas de espectro estendido foram introduzidos como agentes antimicrobianos de primeira escolha no tratamento de infecções causadas por *Salmonella* multidroga resistente (MDR) (SOOD et al. 1999; KARON et al. 2007). Porém, a resistência bacteriana a novas classes de antimicrobianos vem aumentando, incluindo as fluoroquinolonas, quinolonas e cefalosporinas de 3ª e 4ª geração (EL-TAYEB et al., 2017; QIAO et al., 2017). O surgimento de sorotipos de *Salmonella* resistentes a fluoroquinolonas e cefalosporinas representa um novo desafio no tratamento de pacientes infectados, e a falta de uma antibioticoterapia eficaz pode aumentar as taxas de morbimortalidade (SOUZA et al., 2010).

Em *S. enterica*, a resistência antimicrobiana é atribuída a vários mecanismos, como A) modificação do alvo do antibiótico, B) ativação de bombas de efluxo, C) alterações na permeabilidade da membrana, D) além da degradação enzimática (Figura 5) (ROSSI; ANDREAZZI, 2005; EL-TAYEB et al., 2017).

Figura 5 – Mecanismos de resistência microbiana.



Fonte: Autor (2020). Criado com BioRender.com

2.10.1 Modificação do alvo do antibiótico

A alteração do alvo é um dos mecanismos utilizados pelas bactérias para induzir resistência aos antibióticos. A maioria dos antibióticos exerce suas atividades ligando-se a um alvo específico, impedindo sua função e consequentemente matando a bactéria ou inibindo seu crescimento (SANSEVERINO et al., 2018). A modificação do alvo compreende: i) a mutação no gene que codifica o alvo; ii) a modificação enzimática do alvo; e iii) a substituição do alvo clássico (MUNITA ARIAS, 2016, SANSEVERINO et al., 2018). A modificação do alvo atua como um mecanismo de auto-resistência contra várias classes de antibióticos, incluindo β-lactâmicos, glicopeptídeos, macrolídeos, lincosamidas e estreptograminas (MLS) e aminoglicosídeos (PETERSON; KAUR, 2018).

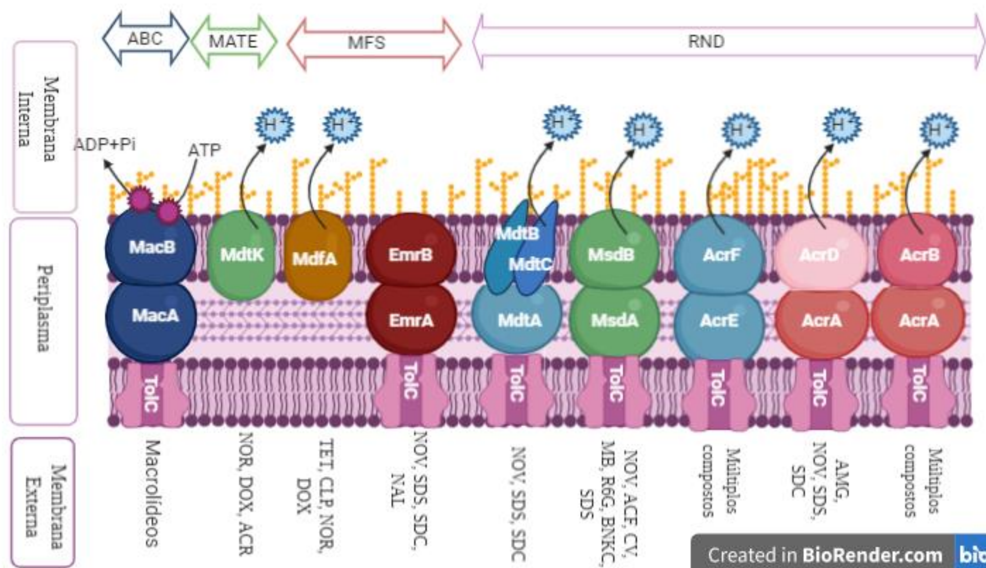
2.10.2 Bombas de efluxo

A remoção ativa de agentes antimicrobianos do interior da célula é um mecanismo de resistência altamente eficiente (WRIGHT, 2011). As bombas de efluxo constituem complexos protéicos que formam um canal pelo qual o antibiótico é expulso para o ambiente externo, diminuindo sua acumulação no interior da célula bacteriana. As bombas de efluxo em bactérias MDR podem ser agrupadas em cinco famílias estruturais diferentes: a superfamília de cassete de ligação de adenosina trifosfato (ATP) (ABC - ATP-binding cassette superfamily), a superfamília de extrusão de compostos tóxicos e multidrogas (MATE – Multidrug and toxic compound extrusion family), a superfamília de facilitadores principais (MFS- major facilitator), a pequena família de resistência multidroga (SMR – Small multidrug resistance family) e a superfamília de divisão celular de nodulação de Resistência

(RND - Resistance nodulation cell division superfamily) (ZHANG et al., 2011). Além da presença de bombas de efluxo, a superexpressão de bombas de efluxo também pode contribuir para a multirresistência em bactérias Gram-negativas. Em bacilos Gram-negativos não fermentadores de lactose, as mutações cromossômicas podem levar à hiperexpressão de bombas de efluxo e resistência (BELLO et al., 2018).

Em *Salmonella* foram descritos nove complexos de bombas de efluxo pertencentes a quatro famílias: família MFS (complexos: EmrAB and MdfA); família RND (complexos: AcrAB, AcrD, AcrEF, MdtABC and MdsAB); família MATE (complexo: MdtK) e família ABC (complexo: MacAB) (ANDERSEN et al., 2015) (Figura 6).

Figura 6 – Famílias de bombas de efluxo encontradas em *Salmonella*.



*família MFS (complexos: EmrAB and MdfA); família RND (complexos: AcrAB, AcrD, AcrEF, MdtABC and MdsAB); família MATE (complexo: MdtK) e família ABC (complexo: MacAB). Abreviações utilizadas na figura significam: Aminoglicosídeos (AMG), Novobiocina (NOV), Dodecil sulfato de sódio (SDS), Acriflavina (ACR), Cristal violeta (CV), Azul de metileno (MB), Rodamina 6G (R6G), Cloreto de benzalcônio (BNKC), ácido nalidixico (NAL), tetraciclina (TET), Cloranfenicol (CLP), Norfloxacina (NOR), Doxorrubicina (DOX) e macrolídeos (MAC). **Fonte:** Adaptado de Andersen et al., 2015 criado com BioRender.com.

2.10.3 Alteração da permeabilidade da membrana

Muitos dos antibióticos têm alvos bacterianos intracelulares ou, no caso das bactérias Gram-negativas, alvos localizados na membrana citoplasmática (membrana interna), assim, o composto deve atravessar a membrana externa e/ou citoplasmática para exercer seu efeito antimicrobiano (MUNITA; ARIAS, 2016). A alteração da permeabilidade da membrana externa bacteriana exerce um papel importante para impedir que o antibiótico alcance seu alvo intracelular ou periplasmático, pois diminui a captação da molécula antimicrobiana através da redução da abertura das porinas, proteínas que compõem a membrana externa e formam canais, regulando a entrada de substâncias atuando assim como a primeira linha de defesa contra a penetração de vários compostos tóxicos, incluindo vários agentes antimicrobianos (MOREIRA et al.,2013).

As alterações das porinas podem ser alcançadas por três processos gerais: (i) uma mudança no tipo de porinas expressas, (ii) uma mudança no nível de expressão das porinas e (iii) comprometimento da função das porinas (MUNITA; ARIAS, 2016). Todos esses mecanismos resultam em uma diminuição da penetração do antibiótico em bactérias e afetam principalmente moléculas como β-lactâmicos, tetraciclinas e algumas fluoroquinolonas, pois tratam-se de moléculas hidrofílicas que frequentemente usam canais de difusão cheios de água, conhecidos como porinas, para cruzar essa barreira e são particularmente afetadas por mudanças na permeabilidade da membrana externa (MUNITA; ARIAS, 2016; SANSEVERINO et al., 2018). A redução da permeabilidade da membrana externa também pode desempenhar um papel importante na resistência às quinolonas e aminoglicosídeos (CESUR; DEMIRÖZ, 2013).

2.10.4 Inativação enzimática do antibiótico

Um dos principais mecanismos de resistência aos antibióticos ocorre devido à capacidade bacteriana de produzir enzimas que degradam ou inativam o antibiótico através da hidrólise, transferência de um grupo químico ou processo de oxirredução (COSTA; SILVA JUNIOR, 2017; SANSEVERINO et al., 2018). Este mecanismo enzimático é bem conhecido em bactérias Gram-negativas e Gram-positivas, e a maioria dos antibióticos afetados por essas modificações enzimáticas exercem seu mecanismo de ação inibindo a síntese de proteínas no nível do ribossomo (MUNITA; ARIAS, 2016). As reações bioquímicas mais frequentes são catalisadas por enzimas que promovem (i) acetilação (aminoglicosídeos, cloranfenicol, estreptograminas), (ii) fosforilação (aminoglicosídeos, cloranfenicol) e (iii) adenilação

(aminoglicosídeos, lincosamidas). Independentemente da reação bioquímica, o efeito resultante costuma estar relacionado ao impedimento estérico que diminui a avidez do fármaco por seu alvo, o que, por sua vez, se reflete em concentração inibitória mínima (MUNITA; ARIAS, 2016, COSTA; SILVA JUNIOR, 2017; SANSEVERINO et al., 2018). Muitos tipos de enzimas foram descritos dentre elas: Enzimas modificadoras de aminoglicosídeos (AME - Aminoglycoside modifying enzymes) que são enzimas que conferem resistência por meio modificação bioquímica dos aminoglicosídeos; cloranfenicol acetiltransferases (CAT - chloramphenicol acetyltransferases) que são capazes de modificar quimicamente o substrato; e um dos melhores exemplos de resistência por hidrólise é representado pelas enzimas β-lactamases. A hidrólise de antibióticos β-lactâmicos pelas β-lactamases é o principal e o mais comum mecanismo de resistência para essa classe de agentes antibacterianos em bactérias Gram-negativas clinicamente importantes. Como penicilinas, cefalosporinas e carbapenêmicos estão entre as drogas preferenciais de escolha no tratamento para muitas doenças infecciosas, a presença e as características dessas enzimas desempenham um papel crítico na seleção da terapia apropriada (BUSH; JACOBY et al., 2010).

2.11 Impactos da Presença de *Salmonella* na cadeia de produção animal

A alimentação e a nutrição constituem requisitos básicos para a proteção e promoção da saúde, possibilitando assim um potencial de qualidade de vida para cada indivíduo (MARCHI et al, 2011). Alimentos de origem animal são ricos em nutrientes como proteínas, lipídios e minerais (COSBY et al., 2015). No entanto, são suscetíveis à contaminação por *Salmonella* durante os procedimentos de produção (XU, 2020).

Surtos causados por alimentos contaminados com *Salmonella* têm ocorrido em todo o mundo. Produtos alimentícios de origem animal são os maiores responsáveis pela distribuição mundial das salmoneloses e suas complicações posteriores. Sua presença em animais, criados com objetivo comercial, aponta a *Salmonella* como o mais incidente e relevante agente etiológico de enteroinfecções. Isso ocasiona perdas de milhões de dólares para a indústria tanto para o mercado interno quanto para exportação, sendo que, em alguns países, a rigidez na inspeção representa necessidade constante de qualidade (BRASIL, 2011). Este fato despertou aos órgãos e autoridades sanitárias dos países atenção sobre a utilidade de medidas para diminuir o risco de transmissão, de doenças através de alimentos, o que moveu a Organização das Nações Unidas para a Alimentação e a Agricultura (FAO) a formar a

Organização Mundial do Comércio, que fez com que os países revisassem suas normas, estratégias e políticas de inocuidade para certificar que a população consuma alimentos que tenham condições sanitárias adequadas, e também garantir o comércio internacional (OPAS, 2001).

Na produção animal, o fator epidemiológico mais destacado nos animais é o estado de portador, animais com infecções subclínicas ou portadores assintomáticos, e as dificuldades técnicas para sua detecção antes ou durante a inspeção dos produtos de origem animal os convertem em fonte contínua de contaminação para o meio ambiente, animais ao redor e para os homens através da cadeia alimentar (BRASIL, 2011; CAETANO; PAGANO, 2019). Santos et al., 2018 relataram que peixes são portadores assintomáticos de *Salmonella* sp., portanto constituem uma fonte de infecção a humanos. Surtos ocasionados pela presença de *Salmonella* em produtos da aquicultura vêm sendo relatado no mundo inteiro (EFSA, 2010, CSPI, 2009, AMAGLIANI et al., 2012, BUDIATI et al., 2013, TRAORÉ et al., 2015, ZHANG et al., 2015; DIB et al., 2018, FERRARI et al., 2019, SANTOS et al., 2019). Segundo Ferrari et al., 2019, surtos de *Salmonella* relacionados ao consumo de peixes podem atingir 12%.

No Brasil, *Salmonell*a spp. está entre os principais agentes envolvidos em surtos de doença transmitida por alimentos (DTA) nos últimos 17 anos, sendo responsável por mais de 30% das DTAs (BRASIL, 2018). Mesmo não sendo notificada em grande parte dos casos às autoridades sanitárias, devido à natureza branda dos sintomas causados por muitos dos patógenos relacionados à DTA (COSTALUNGA; TONDO, 2002; FORSYTHE, 2010; CAETANO; PAGANO, 2019) houve aumento acentuado e contínuo do número de casos vinculados a determinados sorotipos de salmonela no Brasil, os quais variam geograficamente. Além disso, foi observada uma inter-relação entre os sorotipos incidentes entre alimentos de origem animal e de origem humana (RODRIGUES, 2011, 2014). Portanto, o conhecimento da epidemiologia da salmonelose é de fundamental importância para o controle sanitário da doença, sendo o sorotipo o marcador epidemiológico de eleição desse gênero (RODRIGUES, 2011).

Hendriksen et al., 2011, analisaram a distribuição global dos 15 sorotipos de *Salmonella* mais frequentemente isolados de humanos de 2001 a 2007 em laboratórios de 37 países que participaram da Rede Global de Infecções Alimentares da Organização Mundial da Saúde e que demonstraram proficiência em sorotipagem no Sistema de Garantia de Qualidade

Externa da Rede Global de Infecções Transmitidas por Alimentos. Em todas as regiões durante o período de estudo, com exceção das regiões da Oceania e América do Norte, *Salmonella* sorotipo Enteritidis foi o sorotipo mais comum seguido pelo sorotipo Typhimurium. Sorotipo Newport foi observado principalmente na América Latina, América do Norte e países europeus, Virchow principalmente em países asiáticos, europeus e oceânicos, Hadar em países europeus e Agona nos países da América Latina, América do Norte e Europa. Neste mesmo estudo os 20 sorotipos mais comum entre 2000 e 2007 na América Latina, estando Brasil incluso nesta pesquisa, foram Enteritidis, Typhimurium, Typhi, Agona, Parathyphi B, Newport, Infantis, Montevideo, Panama, Saintpaul, Sandiego, Dublin, Corvallis, Anatum, Oranienburg, Javiana, Derby, Braenderup, Heidelberg e Rubislaw.

Em um estudo de meta-análise com objetivo de relatar a distribuição mundial e a ocorrência de diferentes sorotipos em diferentes matrizes de alimentos de origem animal e seu papel como veículos de transmissão de cada um dos sorotipos para humanos, os autores dectectaram que sorotipo *S.* Typhimurium apresentou distribuição cosmopolita, relatada nas matrizes e continentes avaliados. As aves continuam a desempenhar um papel central na disseminação do sorotipo Enteritidis para humanos. Os sorotipos Anatum e Weltevreden foram os mais frequentemente encontrados em carne bovina e produtos da aquicultura, respectivamente. Além disso, recomendaram um monitoramento cuidadoso de certos sorotipos, como Derby, Agona, Infantis e Kentucky, e concluíram que os sorotipos mais frequentemente relatados e as matrizes de alimentos de origem animal constituem os principais veículos para a transmissão desse patógeno, sugerindo que programas de controle podem ser melhorados e intervenções específicas podem ser implementadas na tentativa de reduzir o risco de este patógeno atingir os humanos (FERRARI et al., 2019).

No Brasil, um levantamento epidemiológico realizado pelo Laboratório de Referência Nacional de Enteroinfecções Bacterianas (LRNEB, IOC/FIOCRUZ/RJ) apontou um panorama global dos sorotipos de *Salmonella* spp. circulantes no país entre 2000 e 2009, retratando a incidência de *Salmonella* spp. em diferentes fontes da cadeia epidemiológica, incluindo isolados de fonte humana, alimentar, animal, ambiental, matéria-prima e rações (RODRIGUES et al., 2010). Segundo os autores, os sorotipos detectados através de estudos epidemiológicos e de monitoramento demonstraram oscilações quanto à sua frequência, no entanto se mantêm presentes em níveis variáveis em todas as fontes da cadeia alimentar. Em cepas de origem animal, nos anos de 2008 e 2009, destacaram-se a *S.* enterica sorotipo

Typhimurium, Enteritidis e Schwarzengrund, enquanto que para as provenientes de matéria-prima, rações e ambiente, os índices apontaram a prevalência dos sorotipos Agona, Panama, Minnesota e Senftenberg, envolvendo diferentes regiões do país (RODRIGUES et al., 2010).

No que se diz respeito a *Salmonella* em peixes existem poucos estudos realizados no Brasil (FERNANDES et al., 2018; SANTOS et al., 2018). Alguns estudos têm evidenciado a ocorrência deste patógeno em produtos da aquicultura: na região Nordeste do país, foi relatada a ocorrência de 5% dessa bactéria em peixes e crustáceos em cativeiro, bem como 18,5% em camarão salgado e seco e farinha de peixe, comercializados no varejo da cidade de Belém, no estado do Pará, e a incidência de contaminação de 3,4% em tilápia do Nilo (*Oreochromis niloticus*) também foi relatada no mesmo estado (FERNANDES et al., 2018). Apesar de estes estudos avaliarem a presença de salmonela não foram detectados quais os sorotipos estavam presentes. Santos et al. 2018, detectaram a presença dos sorotipos Brandenburg, Hadar, Heidelberg, Panama e Saintpaul, em pisciculturas com produção de peixes nativos. Para avaliar quais sorotipos estão envolvidos no setor para que estratégias sejam traçadas é necessário o desenvolvimento de estudos epidemiológicos nesta cadeia de produção.

Além dos alimentos de origem animal serem uma das maiores fontes de *Salmonella* spp., tem havido preocupação sobre resistência antimicrobiana em *Salmonella*. Tal fato tem sugerido que o uso de antimicrobianos na produção animal pode contribuir para a presença de resistência antimicrobiana em *Salmonella* spp. que infectam humanos (Cruchaga et al., 2001; Iovine e Blaser, 2004). A incidência de resistência bacteriana a antimicrobianos representa risco ao meio ambiente, à saúde humana e animal. Esse tema de extrema importância tem sido objeto de atenção de instituições como a Organização Mundial da Saúde – OMS, o Escritório Internacional de Epizootias – OIE e o Codex Alimentarius, que vêm discutindo soluções globais para o problema. Tal fato tem por base sua distribuição mundial, sendo detectada na maioria das espécies animais utilizada para consumo humano, além de animais silvestres e domésticos (BRASIL, 2011).

Visto que o Brasil é um importante exportador mundial de carnes, medidas cada vez mais rígidas de controle sanitário devem ser estabelecidas, a fim de impedir prejuízos referentes a perdas indiretas ocasionadas por problemas sanitários.

2.12. Métodos Moleculares

A presença de salmonela circulante em produtos de origem animal destinado a alimentação humana e sua interação com ambiente é um problema de saúde pública. Além disso, os isolados de *Salmonella* são diversos na resistência antimicrobiana tanto fenotipicamente quanto geneticamente. Portanto, a prevalência e a gravidade de *Salmonella* MDR são de grande preocupação porque os níveis de resistência são variados e influenciados pelo uso de agentes com ação antimicrobiana em humanos e animais. Sendo assim investigar melhor a prevalência de *Salmonella* e responder às emergências de segurança alimentar de forma rápida e eficaz é muito importante analisar a relação entre os isolados usando tecnologias como avaliação através de métodos moleculares que nos confere respostas rápidas, quando comparado com o método tradicional de cultura bacteriana, utilizado na rotina de laboratórios clínicos, mais amplas, uma vez que os genes da bactéria são conhecidos, é possível identificar sua origem, fontes da infecção, fatores de virulência, presença de contaminação cruzada no processo, resistência a antimicrobianos, capacidade de formação de biofilme, nos auxiliando no entendimento do comportamento da *Salmonella* (XU et al., 2020).

A reação em cadeia da polimerase (Polymerase Chain Reaction - PCR) é um método altamente sensível, por meio da qual, pequenas quantidades de sequências de DNA ou RNA específicas podem ser enzimaticamente amplificadas até que sejam obtidas milhões de cópias da sequência alvo (KONEMAM et al., 2001). A PCR tem nos auxiliado na identificação de microrganismos e na identificação de elementos genéticos móveis que auxiliam na transferência de genes de resistência intra e interespecies. A disseminação de genes de resistência a antimicrobianos entre microrganimos tem sido facilitada pela presença de elementos como integrons via transferência horizontal de genes, o qual desempenha um papel importante na evolução e adaptação bacteriana, abrangendo novos mecanismos de resistência contra múltiplos agentes antimicrobianos. Os integrons são principalmente sistemas de recombinação específica do local com a capacidade de extirpar ou integrar genes de resistência a atimicribianos (KAUSHIK et al., 2019). Estudos têm mostrado que integrons de classe 1 são comuns entre *Salmonella* MDR. A maioria desses integrons contem genes de resistência que podem ser facilmente transmitidos para outros microrganimos. Assim, genes de resistência idênticos são encontrados em cepas humanas, alimentares e ambientais, sugerindo ainda ampla disseminação da resistência a múltiplas drogas em *Salmonella* (KAUSHIK et al., 2018).

Outro método molecular é o sequenciamento do genoma completo (Whole Genome Sequencing - WGS), que é eficiente e oferece vários benefícios para um estudo completo de monitoramento de origem, dispersão e correlação de isolados de *Salmonella* quando combinadas com ferramentas de bioinformática (ALLARD et al., 2018; BROWN et al., 2019; TANG et al., 2019; PEARCE et al., 2020; HASSENA et al., 2021). O sequenciamento do genoma está revolucionou o campo da microbiologia por meio do desenvolvimento de várias ferramentas baseadas em sequência. WGS proporciona a detecção molecular, ensaios sorológicos, de genotipagem e fornece uma abordagem fácil para estudar genes de virulência e de resistência aos antimicrobianos (HASSENA et al., 2021). CDC tem usando WGS para investigações de surto de *Salmonella* (CDC, 2016). WGS tem sido utilizado cada vez mais em investigações de incidentes de contaminação na indústria de alimentos, principalmente porque o custo continua diminuindo e a facilidade de uso tem aumentado. WGS também tem uma série de aplicações adicionais na área indústria de alimentos, como o monitoramento de ingredientes, suprimentos, identificação da persistência microbiana no ambiente de processamento, presença de bactéria com resistência antimicrobiana, facilitando a melhoria da gestão sanitária, microbiana, controle de perigos e avaliação de risco microbiológico o que impulsionará ainda mais a implementação dessa ferramenta (ALLARD et al., 2018; TANG et al., 2019).

O WGS oferece informações no qual é possível entender a epidemiologia do patógeno e estabelecer parâmetros para avaliar os riscos à saúde pública. Este monitoramento contribui na garantia da segurança do alimento produzido, podendo inclusive, ser utilizado no futuro, como marcador das estirpes circulantes no país, o que ajudaria a entender problemas relacionados à sua origem ou às variações na sua patogenicidade.

3 OBJETIVOS

3.1 Objetivo Geral

Isolar *Salmonella* spp. de pisciculturas na região de Dourados/MS, determinar os sorotipos, avaliar o perfil de suceptibilidade a antibióticos e seus possíveis mecanismos de transmissão de resistência.

3.2 Objetivos específicos

1) Avaliar a presença de *Salmonella* em água de tanque, biofilme epilítico e peixe nas pisciculturas na região de Dourados/MS;
2) Identicar sorotipos de *Salmonella* isolados em pisciculturas;
3) Avaliar o perfil de susceptibilidade antimicrobiana das *Salmonella* spp.;
4) Determinar a presença de Integrons nos isolados estudados, identificando a classe e sua frequência;
5) Sequenciar isolado de interesse para avaliar o perfil genotípico de resistência a antimicrobianos e virulência;
6) Avaliar a utilização de um sistema de monitoramento da presença de *Salmonella* em pisciculturas por meio de dispositivos para formação de biofilme epilítico.

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5 APÊNDICES

5.1 Manuscrito I: Presence and antimicrobial susceptibility of *Salmonella* spp. from fish farms

Manuscrito nas normas para submissão no periódico "International Journal of Food Microbiology".

Link para as normas da revista: https://www.elsevier.com/journals/international-journal-of-food-microbiology/0168-1605/guide-for-authors

Presence and antimicrobial susceptibility of *Salmonella* spp. from fish farms

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Abstract

Salmonella ssp. are important foodborne pathogens worldwide, frequently responsible for infectious outbreaks through consumption of contaminated food. Aquaculture products can become sources of *Salmonella* by exposure to contaminated water or through processing practices, thus representing a public health hazard. Therefore, this study aimed to evaluate presence and serotypes, assess integrons, and characterize antimicrobial resistance profiles of *S. enterica* in fish farms. A total of 90 samples of fish, pond water, and epilithic biofilm, were collected from aquacultural facilities. *Salmonella* isolates were identified and serotyped by slide agglutination according to Kauffmann–White. Antimicrobial susceptibility was evaluated using Kirby Bauer disk diffusion, and the presence of integrons was identified using *Polymerase Chain Reaction* (PCR). Thirteen *S. enterica* isolates were isolated and identified as *S.* Minnesota, *S.* Panama, *S.* Anatum, and two isolates did not have the serotype identified (*Salmonella enterica* subsp. *enterica*). We observed high resistance to sulfonamides (100%), trimethoprim (84.61%), tetracycline (46.15%) and streptomycin (46.15%), all antibiotics classified by the World Health Organization as important for human medicine. Multiple antibiotic resistance was confirmed in 84.61% of the isolates, 100% exhibited class 1 integrons and 7.69% exhibited class 2 integrons, limiting therapeutic options and representing a potential health hazard. This study provided data on the presence, serotypes, antimicrobial resistance and presence of the integrons of *Salmonella* in fish farm, and indicated the need for monitoring programs for microbiologic safety in such projects and for more prudent drug use in aquaculture production in order to reduce the risk of development and spread of antimicrobial resistance.

Keywords

Salmonella; Aquaculture products; Serotyping; Antimicrobial resistance; Public health

1.Introduction

Animal-derived meat rich in nutrients such as protein, lipids and minerals, is a very desirable food for humans. However, it is susceptible to contamination during the procedures of production (Xu et al., 2020). Similar to other sectors of livestock production, fish production uses intensive and semi-intensive practices, characterized by high stock density and volume; heavy use of formulated feeds containing antibiotics, antifungals and other pharmaceuticals; heavy use of pesticides and disinfectants; consortium with other animals; use of tank fertilization such as manure use; use of agricultural by-products in fish feed; introduction of drugs to control or prevent pathogens; and use of growth promoters (Cabello et al., 2016; Fernandes et al., 2018; Sapkota et al., 2008). Current aquaculture practices could thus lead to exposures chemicals and biological agents. Freshwater products, in particular, are reported as vehicles for foodborne transmission of pathogens, such as *Salmonella* (Fernandes et al., 2018; Sapkota et al., 2008).

Salmonella spp, members of the Enterobacteriaceae family, are Gram-negative, rod-shaped bacteria that cause salmonellosis. *Salmonella* spp. are categorized into two species (*Salmonella enterica* and *S. bongori*), six subspecies, and >2600 serotypes (Issenhuth-Jeanjean et al., 2014). Different serotypes are responsible for subclinical, clinical, severe infections in farm and pet animals, as well as typhoid fever and gastroenteritis in humans worldwide, including developed and industrialized countries (Evangelopoulou et al., 2015; Weinberger and Keller, 2005). *S. enterica* is an important human and animal pathogen, commonly transmitted to humans through contaminated food and water, direct contact with animals or, more indirectly, through environmental pathways (Eng et al., 2015, Michael and Schwarz, 2016). In general, food animals such as swine, poultry and cattle are prime sources of *Salmonella* infections (Eng et al., 2015). The incidence of salmonellosis caused by fish consumption has become a concern for public health agencies in several countries, in

recognition of significant increases in consumption of aquaculture products (Zhang et al., 2015; Fernandes et al., 2018). Further, aquatic foods are often consumed raw, what increases pathogen exposure risk (Fernandes et al., 2018). Thus, *Salmonella* remains a formidable public health challenge (Cummings et al., 2012) with reported increases in incidence (CDC, 2013) and antibiotic resistance (Eng et al., 2015) and thereby may play an important role in the dissemination of antimicrobial resistant isolates throughout the food chain. Food animals represent the major reservoir for the transmission of antimicrobial resistant *Salmonella* into humans. In addition, the incorporation of resistance genes in human bacteria threatens the efficiency of human antibacterials (Hassena et al., 2021).

The presence of genetic elements such as integrons has contributed to the rapid transmission of drug resistance in bacterial pathogens, especially among members of the Enterobacteriaceae family. Integrons are mobile DNA elements capable of detention and excision of genes, particularly those responsible for antibiotic resistance (Malek et al, 2015).

The continuous change in the distribution and antimicrobial resistance profiles of *Salmonella* serotypes in food of animal origin poses a challenge for salmonellosis control. This is further exacerbated by increasing global trade of food products of animal origin, which facilitates the dissemination of new and antimicrobial resistant serotypes. Therefore, continuous surveillance of the distribution and antimicrobial resistance profiles of *Salmonella* serotypes is crucial to identify the sources of infection, detect outbreaks, and implement prevention and control measures (Mechesso et al., 2020). In a One Health perspective, the aquaculture industry has contributed importantly to the emergence and dissemination of antimicrobial resistant bacteria and antimicrobial resistance genes (Martins et al., 2019). Thus, the aim of this study were to evaluate the presence of *Salmonella* in fish farms, identify serotypes and characterized antibiotic resistance profiles.

2. Material and methods

2.1. Sample collection

Three fish farms, located in the region Dourados, Mato Grosso do Sul, Brazil (Fish farm 1- Fish, Lambari (*Astyanax lacustris*) were raised in monoculture); (Fish farm 2 – Fish were raised in polyculture systems, with four native species in the same earth pond: Tambaqui (*Colossoma macropomum*), Pacu (*Piaractus mesopotamicus*), Dourado (*Salminus brasiliensis*) and Patinga (*Piaractus mesopotamicus*)); (Fish farm 3 –Fish, Nile Tilapia (*Oreochromis niloticus*), were raised in monoculture) were evaluated in this study.

Sampling occurred from October 2017 to May 2018. Ninety samples were collected, including fish (24), water (48), and epilithic biofilm (18). Epilithic community samples were obtained using Polyethylene terephthalate (PET) slides, installed 30 days before collection. On the day of collection, e*pilithic* slides were placed in a sterile polypropylene bag. Water samples were collected from fish tanks in sterile bottles, submerging containers to 20 cm. Fish samples were randomly selected from tanks with the aid of fishing net. Fish were anesthetized with eugenol (50 mg/L) and placed in sterile polypropylene bags. After collection, the samples were placed in polystyrene boxes containing ice; temperatures were between 4 and 8°C during transportation. The samples were delivered and analyzed in the laboratory within eight hours. Study protocols were approved by the Ethics Commission on the Use of Animal Use from Federal University of Grande Dourados (CEUA-UFGD) under process number 20/2018.

2.2. *Salmonella* isolation and identification

Isolation of *Salmonella* spp. was accomplished using three methods. In method A, fish samples were placed in sterile plastic bags and rinsed with 250 mL of buffered Peptone Water

(BPW) (Oxoid, Baringstoke, Hampshire, UK) following the protocol outlined in the U.S. Department of Agriculture (USDA)/Food Safety and Inspection Service (FSIS) (2017) and ISO 17604:2015. In method B, aliquots of 25 mL of water samples were added to 225 mL of BPW. In method C, samples of *epilithic* biofilm slides were placed in a sterile plastic bag containing 25 mL of the BPW and removed using a sterile razor blade.

The detection of *Salmonella* was based on standard methods (ISO 6579:2002/Amd.1:2007). For pre-enrichment, BPW was incubated at 37 °C for 18-24 h. Thereafter, 0.1 mL of pre-enriched sample was added to 10 mL of Rappaport-Vassiliadis (RV) broth (Merck, KGaA, Darmstadt, Germany), and another 1 mL of the same sample was transferred to 10 mL of tetrathionate (TT) broth (Merck KGaA, Darmstadt, Germany). Broth cultures were incubated at 42 °C and 37 °C respectively for 24 h. Following incubation, a loopful of RV and TT enrichment broth culture was streaked onto xylose-lysine-tergitol agar (XLD) (Merck KGaA, Darmstadt, Germany) plates. Plates were incubated at 37 °C for 24 h. Isolated colonies giving typical reactions were purified by streaking onto nutrient agar plates (Merck KGaA, Darmstadt, Germany) and subjected to the biochemical tests.

2.3. Serotyping of *Salmonella*

The serotyping used slide agglutination according to Kauffmann–White. All isolates were serotyped at the Brazilian National Reference Laboratory of Enterobacteria of the Oswaldo Cruz Foundation, Brazilian Ministry of Health.

2.4. Antibiotic susceptibility testing

Antimicrobial susceptibility was evaluated using Kirby and Bauer disk diffusion for 14 antimicrobial agents, in accordance with Clinical Laboratory Standards Institute guidelines (CLSI, 2012). Antimicrobials evaluated were ampicillin 10 µg (AMP), amoxicillin-clavulanic acid 20/10 µg (AMC), cefotaxime 30 µg (CTX), ceftazidime 30 µg (CAZ), cefoxitin 30 µg

(FOX), ceftriaxone 30 µg (CRO), chloramphenicol 30 µg (C), tetracycline 30 µg (TE), nalidixic acid 30 µg (NA), ciprofloxacin 5 µg (CIP), gentamicin 10 µg (CN), streptomycin 10 µg (S), trimethoprim 5 µg (W) and sulfonamide 300 µg (SX) (Oxoid, Basingstoke, United Kingdom). *Escherichia coli* (ATCC 25922) was used as quality control strain. Diameters of zones of inhibition around discs were measured and compared against recommendations of the CLSI to classify the strains as resistant, intermediate, or sensitive to a specific antibiotic (CLSI, 2019). *Salmonella* isolates resistant to three or more classes of antibiotics were defined as multidrug resistant (MDR) according to the definition of the National Antimicrobial Resistance Monitoring System (NARMS) (FDA, 2014). We also examined the isolates for AmpC or extended-spectrum β-lactamases (ESBL) production using the combination disk method (Jalier et al., 1988).

2.5. PCR screening for integrons

DNA was extracted and the presence of class 1 and 2 integrons was examined by PCR for integrase gene, *intI1,* with primers: F – CAGTGGACATAAGCCTGTTC and R – CCCGACGCATAGACTGTA, and integrase gene *intI2,* F – TTGCGAGTATCCATAACCTG and R – TTACCTGCACTGGATTAAG according to Corrêa, et al., 2014.

3. Results

3.1. *Salmonella* isolation and serotyping

Salmonella spp. were isolated from the three fish farms. Fish Farms 1 and 2 showed presence *Salmonella* only on epilithic biofilms. In contrast, *Salmonella* was found in fish farm

3 in pond water and fish (Table 1). *Salmonella* was isolated from 13 (14.44%) of the total of 90 samples examined−epilithic biofilm (n = 5), fish (n = 3), and pond water (n = 5) samples

The 13 isolates were classified into three distinct *S. enterica* serotypes, Anatum (n = 1), Minnesota (n = 6), and Panama (n = 4). Two isolates did not have the serotype identified (*Salmonella enterica* subsp. *enterica*). Distribution of *Salmonella* serotypes in the different samples and fish farms is presented in Table 1.

3.2. Antibiotics susceptibility in *S. enterica*

Antibiotic resistance among *Salmonella* isolated from fish, pond water and epilithic biofilm samples showed that all isolates were susceptible to cefotaxime, cefoxitin, ceftriaxone, and gentamicin, and resistant to sulfonamide (Table 2). A high proportion of isolates (30.76%) showed intermediate resistance to ciprofloxacin. Highest rates of resistance were detected for sulfonamides (100%), trimethoprim (84.61%), and tetracycline and streptomycin (46.15%). Eleven isolates (84.61%) were considerate MDR, and 11 multi-resistance patterns were identified. The predominant MDR pattern (Table 1) was resistance to streptomycin, trimethoprim and sulfonamides (S+W+SX). None of the *Salmonella* isolates showed Amp C or ESBL phenotype.

3.3. Incidence of class 1 and class 2 integrons in *S. enterica* isolates

The class 1 integrase gene, *intI*1, was identified by PCR in all isolates of *Salmonella* (100%). All *IntI*1 positive isolates showed resistance to two or more classes of antibiotics. Among 13 tested *Salmonella* isolates, only one isolate (7.69%) displayed class 2 integrons, *intI*2, serotype Minnesota (Table 1).

4. Discussion

The occurrence of *Salmonella* in fish, seafood and fish farms has been documented in several countries around the world (Amagliani et al., 2012, Budiati et al., 2013, CSPI, 2009, Dib et al., 2018, EFSA, 2010, Traoré et al., 2015, Santos et al., 2019, Zhang et al., 2015). The presence this pathogen in all fish farms studied to date suggests fecal contamination, since these bacteria are natural inhabitants of human and animal intestines. They are not naturally present in the aquatic environment or normal microbiota of aquatic animals (Dib et al., 2018). The presence of these microorganisms in fish farms is likely due to fecal pollution of surface water from human and animal sources, organic material, and overstocking of fish (Amagliani et al., 2012).

All serotypes (Anatum, Minnesota and Panama) isolated in this study have been identified in poultry, swine, fish, seafood and other livestock animals. The unique serotype found in more than one fish farm was Panama. This serotype usually causes gastrointestinal infection in humans but is more widely known for causing invasive disease and colonizing extraintestinal sites. This serotype has been isolated from diverse sources including human and non-human, such as chicken, fish, pork and water (Carneiro et al., 2019, Fernandes et al., 2018, Pulford et al., 2019, Santos et al., 2019). Serotype Minnesota was unique found in fish samples in this study. This serotype is a foodborne pathogen mainly associated with the poultry supply chain where it colonizes the gastrointestinal tract of poultry and consequently can be disseminated to the environment, humans and other animals (Moura et al., 2017). Conversely, Serotype Anatum was found only in epilithic biofilm samples. This serotype constituted the largest proportion of isolates among non-human sources and it is a serotype regarding foodborne illnesses and has caused outbreaks involving various food sources (Fernandes et al., 2018, Ferrari et al., 2019).

Lunestand et al., 2007 and Santos et al., 2019 suggest that fish feed may be a gateway to *Salmonella* in fish farm. Products of poultry chains are used in aquaculture as fertilizer in culture tanks to stimulate the production of algae (FAO 2010, Fernandes et al., 2018); and meat meal, bone meal, blood meal, feather meal and poultry viscera meal are common proteins used as feed ingredients for fish (Furuya and Furuya, 2010). Therefore, *Salmonella* serotypes found in poultry can be transmitted to fish farms by these by-products.

The higher occurrence of *Salmonella* at fish farm 3 may be due to the use of poultry litter as fertilizer in culture tanks and high density stock ponds. Fish then become potential vehicles for *Salmonella* transmission to animals, humans, and environments (Cabello et al., 2013, Iwamoto et al., 2010). In this study, as well as in Budiati et al., 2013 and Santos et al., 2019 cold-blooded animals, such as fish, are possible hosts and passive *Salmonella* transporters that can excrete bacteria without apparent symptoms or clinical manifestations.

Salmonella in fish farm may present an emerging risk to public health. Aquatic food products are often consumed raw or prepared in a way that does not kill bacteria, thus increasing the risk of infection. Santos et al., 2019 reported several cases of *Salmonella* detected in fish slaughterhouses audited by the Brazilian Federal Service of Inspection. According to technical standards for microbiological analysis of food in Brazil, when a batch of products (fresh, frozen meat or processed products) is positive for *Salmonella*, it cannot be marketed and must be discarded and, when disposal occurs for sanitary reasons, producers are not reimbursed (Brasil, 2001). Further, *Salmonella* presence on fish surfaces facilitates cross-contamination during fish processing (Amagliani et al., 2012). An important factor regarding *Salmonella* cross-contamination is that microorganisms remain viable on food contact surfaces for significant periods. This viability is due to the formation of biofilms, a bacterial mode of survival that protects bacteria from stressful environmental conditions, such as drying and cleaning (Fernandes et al., 2018).

The occurrence of the *Salmonella* in epilithic biofilm from fish farms demonstrated that the bacterium can form biofilm on a wide variety of contact surfaces. Cells comprising biofilm can survive long term with resistance to stress, such as desiccation and antibiotics (Fernandes et al., 2018). This ability is critical because it allows *Salmonella* to adapt to multiple conditions, such as soil and aquatic environments, and survives long enough for efficient passage into new hosts (Amagliani et al., 2012; Budiati et al., 201. Cabello et al., 2013 suggested that biofilm epilithons of aquacultural structures represent conditions in aquatic environments that favor horizontal gene transfer.

Another risk factor to public health is the widespread use of antimicrobial drugs in fish farming, and the related risk of the emergence and spread of resistance among human pathogens (Serrano, 2005; Zhang et al. 2015). In animal husbandry, including aquaculture, antibiotics are widely used prophylactically and metaphylactically. Also, their use as growth promoters in subtherapeutic doses has contributed to promoting the development of resistance (Serrano, 2005, Cabello et al., 2013). The differences in levels of antimicrobial resistance and decreasing susceptibility may be a result of the common use of antimicrobials. Increased antimicrobial use in fish farms may create a selective pressure for higher levels of antimicrobial resistance.

Antimicrobial agents tested in the present study are commonly used in aquaculture; several are also used in human medicine and classified by the WHO as critically important for human use (Heuer et al., 2009). Among antimicrobials listed are sulfonamides, tetracycline and streptomycin, for which isolates showed greater resistance. High incidence of resistance to sulfonamides, tetracycline, trimethoprim and streptomycin observed in the present study might be associated with their use frequent in aquaculture feed and their use present at therapeutic or subtherapeutic levels to prevent bacteriosis (Zhang et al., 2015). In Brazil,

oxytetracycline is approved for use in aquaculture and tetracycline is among the most sold and used veterinary antimicrobial agents (EMA, 2015).

However, in Brazil, use of unregistered antibacterials ("*off label*") for fish farming is a relatively common practice, although illegal. Sulfonamides and fluoroquinolones are among the most used (Leal et al., 2017). This use could explain resistance to sulfonamide in all isolates and reduced susceptibility to fluoroquinolones. The emergence of ciprofloxacin resistance in isolates is of public health significance because quinolones are first choice drugs for treatment of infections caused by *Salmonella* spp. in humans (Zhang et al., 2015). In addition, sulfonamide resistance in all *Salmonella* isolates could be linked to presence of class 1 integron. Class I integron possess two conserved sequences (5'CS and 3'CS) separated by a variable region including the gene cassettes integrated with antibiotic resistant genes.The 3′-conserved segment (3′ CS) possesses genes *qacEΔ1* and *sul1*, encoding resistance to quaternary ammonium salts and sulfonamide, respectively (Deng et al., 2015).

Integrons are DNA elements capable of capturing and mobilizing exogenously functional gene cassettes, permitting rapid adaptation to selective pressure (Deng et al., 2015). The high frequency of MDR among *Intl*1 positive isolates is consistent with an association between the presence of class 1 integrons and emerging MDR in *Salmonella*. Class 1 integrons are the most commonly reported among *Salmonella* clinical isolates with variable prevalence among countries and sources (Argüello et al., 2018). These integrons are the most common type identified in MDR *Salmonella*, and plays a critical role in the dissemination of resistance genes among pathogens, such *Salmonella* (Kaushik et al., 2018). Class 1 integrons are associated with a variety of resistance gene cassettes, which confer resistance to antibiotics such as ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline, which were used as first-line drugs for the treatment of salmonellosis (Mthembu et al., 2019). The most frequent resistance patterns to streptomycin, trimethoprim and sulfonamides in this

work may be associated with class 1 integrons Trimethoprim resistance determinants are also detected frequently and trimethoprim + sulfamethoxazole have been a frequently used therapeutic combination (Deng et al., 2015).

Class 2 integrons are also an important vehicle for transmitting resistance genes in *S. enterica* strains. However, class 2 integrons are reported with low occurrence and prevalence compared to class 1 integrons (Deng et al., 2015, Kaushik et al., 2018) and their presence in *Salmonella* isolates is only reported in a few studies (Argüello et al., 2018). Moreover, this class was detected in this study. Due to the fact that the integrons may be horizontally disseminated and that *Salmonel*la antibiotic susceptibility does not have a homogeneous distribution (neither geographical nor temporal), these data provide additional insights into global mobility and genomic plasticity, which contribute to persistence of strains along food chain

Thus, the occurrence of resistance to antimicrobials in *Salmonella* severely limits therapeutic options. Further, antimicrobial resistance can spread among bacterial populations. In order to support the current knowledge regard the epidemiological distribution of MDR strains between the food-animal-environmental interface, our results provide valuable information related to distribution of multidrug-resistant *S. enterica* serotypes in food-producing animal settings. These considerations suggest that excessive aquacultural use of antimicrobials may potentially have major effects on animal and human health and on the environment more broadly, and, therefore, the use of antimicrobial agents in animals should be controlled or avoided to prevent the spread of drug resistance. A One Health approach including multiple interventions is necessary to better understand, prevent, and control of *Salmonella* and its infections.

5. Conclusions

This study provides data of the occurrence of *Salmonella* in fish farm and shows an emerging problem for possible human foodborne infections associated with aquaculture. *Salmonella* was detected in fish farms, in water, in epilithic biofilm and in fish. The fish are as asymptomatic carriers that may pose a risk to humans when contaminated fish are consumed. Fish may pose a risk in serving as reservoirs for antibiotic resistant *Salmonella*. Many of the *Salmonella* isolates were resistant to multiple antimicrobials, and multidrug resistance. Also, the presence of the eleved number of MDR *Salmonella* strains and class 1 integrons from fish farm appears to be an emerging problem. Salmonella can be transmitted by a wide variety of food products and environmental sources. Thus, transmission of *S. enterica* provides a compelling example of the One Health paradigm, with reservoirs of pathogens in humans, animals and the environment. This study underlines the need for integrated surveillance of antibiotic resistance that considers isolates not only from human disease, but also from animal reservoirs and food sources, for monitoring the development and dissemination of antimicrobial resistance. Such information is required in order to make science-based public health policy and to develop effective intervention strategies to ensure the safety of our food supplies.

Declaration Interest

None

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Table 1. Place, source, serotypes, resistance patterns, and presence of class 1 and 2 integrons in *Salmonella* spp. from fish farms

Fish Farm	Samples	Serotype	Antibiotic Resistance pattern	N°. of Antibiotics (Classes)	Integron Class
1	*Epilithic biofilm*	Anatum	AMC+C+TE+W+SX	5(5)	1
	Epilithic biofilm	Panama	AMP+AMC+TE+SX	4 (3)	1
	Epilithic biofilm	Panama	S+W+SX [b]	3 (3)	1
	Epilithic biofilm	Panama	S+W+SX [b]	3 (3)	1
2	*Epilithic biofilm*	-	W+SX	2 (2)	1
3	Fish	Minnesota	S+SX	2 (2)	1
	Fish	Minnesota	S+W+SX [b]	3 (3)	1
	Fish	Minnesota	AMP+AMC+S+W+SX	5(3)	1 and 2
	Pond water	Minnesota	AMC+TE+S+W+SX	5(4)	1
	Pond water	Minnesota	CAZ+W+SX	3 (3)	1
	Pond water	Minnesota	C+ TE+NA+ W+SX	5(5)	1
	Pond water	-	C+TE+W+SX	4 (4)	1
	Pond water	Panama	CAZ+C+TE+W+SX	5(5)	1

Source abbreviations are as follows:

[a]Antimicrobial agents: ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cefotaxime (CTX), ceftazidime (CAZ), cefocitin (FOX), ceftriaxone (CRO), chloramphenicol (C), tetracycline (TE), nalidixic acid (NA), ciprofloxacin (CIP), gentamicin (CN), streptomycin (S), trimethoprim (W), and sulfonamide (SX).

[b]Predominant MDR pattern

Table 2. Antimicrobial resistance of *Salmonella* isolates from fish farms

	N° of resistant isolates (%)/intermediate (%)			
Antimicrobial agents	*Epilithic* biofilm (N = 5)	Fish (N = 3)	Pond water (N = 5)	Total (N = 13)
B-Lactams				
Ampicilin	1 (20)/1 (20)	1 (33.33)	1 (20)	3 (23.07)
Amoxicillin-Clavulanic Acid	2 (40)	1 (33.33)	0(0)/1 (20)	3 (23.07)
Cefotaxime				
Ceftazidime			2 (40)	2 (15.38)
Cefoxitin				
Ceftriaxone				
Phenicols				
Chloramphenicol	1(20)/1(20)		3 (60)	4 (30.76)
Tetracyclines				
Tetracycline	2 (40)		4 (80)	6 (46.15)
Quinolones and Fluoroquinolones				
Nalidixic Acid			2 (40)/2 (40)	2 (15.38)
Ciprofloxacin	0(0)/3(60)		0(0)/1 (20)	0(0)/4(30.76)
Aminoglycosides				
Gentamicin				
Streptomycin	2 (40)/3(60)	3 (100)	1 (20)/4 (80)	6 (46.15)
Trimethoprim				
Trimethoprim	4 (80)	2 (66.66)	5(100)	11 (84.61)

Sulfonamides				
Sulfonamide	5 (100)	3 (100)	5(100)	13 (100)
MDR				
0				
1–2	1(20)	1 (33.33)		2 (15.38)
3–5	4(80)	2 (66.66)	5 (100)	11 (84.61)

MDR, multidrug resistant

5.2 Manuscrito II: Genomic characterization of *Salmonella enterica* serotype Panama isolated from fish farm of Tilapia (*Oreochromis niloticus)*

Manuscrito nas normas para submissão no periódico “Aquaculture”

Link para as normas da revista: https://www.elsevier.com/journals/aquaculture/0044-8486?generatepdf=true

Genomic characterization of *Salmonella enterica* serotype Panama isolated from fish farm of Tilapia (*Oreochromis niloticus)*

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Abstract

Aquaculture tilapia (*Oreochromis niloticus*) production with is currently common practice in production systems of many parts of the world. Unfortunately, this production has been accompanied by rearing practices which result in introduction of hazardous polluting, such as *Salmonella* and antimicrobial agents. *Salmonella* ssp. are important foodborne pathogens worldwide and strains with pattern of the resistance antibiotic, heavy metal and biocide have been identified and are often of zoonotic origin, acquiring resistance in animals and subsequently being passed on to humans through the animal production chain. Despite their broad distribution, there are few records about genetic characterization of *Salmonella* in aquaculture. With the progress of sequencing technologies, it became possible to realize the genetic characterization and to detect the multiple molecular mechanisms leading to antibiotic resistance, biocide and heavy metal resistance, and to detect the virulence factors using whole genome sequencing data. This method has been used in Health and Human Services' agencies for providing a large amount of information that helps agencies control this pathogen in between the food-animal-environmental interface. In this study, we conduct a genome-scale investigation of *Salmonella enterica* serotype Panama from fish farm of *Oreochromis niloticus*. Whole genome sequencing of *Salmonella enterica* serotype Panama was performing using Illumina NextSeq® platform. The genome size was calculated at 4,719,885 bp and 4,525 protein-coding sequences were identified. The *S.* Panama strain belongs to the sequence type ST2041 and displays resistance to aminoglycosides (*aac(6')-Iaa*) as well as genes encoding multidrug efflux pumps and those conferring resistance to biocides and heavy metals. In addition, the strain harbors genes encoding diverse virulence factors, such as adherence, invasion, iron uptake, motility, regulation, secretion systems, and toxins, suggesting potential pathogenic mechanism. Our findings demonstrated that whole genome sequencing and subsequent data analysis provided an accurate tool for genetic

characterization of *Salmonella* in fish farm. These data suggest the putative role of *S.* Panama as a reservoir for biocide and heavy metal resistance genes in aquaculture reflecting the selective pressure imposed by a combination of varied and uncontrolled rearing practices.

Keywords

Aquaculture; *Salmonella* Panama; heavy metal resistance; biocide resistance; virulence genes; whole genome sequencing

1. Introduction

Aquaculture is the fastest growing sector in animal production over the last few decades (Santos et al., 2019). In 2018, it accounted for 46% of the total fish production, amounting to 179 million tons (FAO, 2020). A similar trend was observed in Brazil, which is currently 12th among the world's leading aquaculture producers and 2nd in South America (Martins et al., 2019). Unfortunately, the impressive development of aquaculture has been accompanied by the misuse of antimicrobial drugs. In a One Health perspective, the aquaculture industry has contributed importantly to the emergence and dissemination of antimicrobial resistant bacteria and antimicrobial resistance genes (Martins et al., 2019). The aquaculture sector contributes to the antimicrobial resistance reservoir mainly by administering therapeutic and prophylactic antibiotic treatments to animals but also to use of non-antibiotic chemicals such as biocides and heavy metals which has been shown to increase antimicrobial resistance (AMR) (Cabello et al., 2013; Cheng et al., 2019; Reverter et al., 2020). Unabsorbed and excreted antimicrobial drugs may remain in water columns or accumulate in the sediments, where they can be carried by water currents to sediments at distant sites continuously adding to a richer resistome (Cabello et al., 2013; Han et al., 2017; Martins et al., 2019). As a result, environmental contamination with antibiotics, together with biocides and heavy metals, has resulted in an increasing number of reports of multidrug resistant *Salmonella* serotype (Fernandes et al., 2018).

Salmonella enterica serotype Panama is a nontyphoidal *Salmonella* (NTS) species that is responsible for gastroenteritis and invasive extraintestinal infections in humans, including septicemia, pharyngeal infections, Bartholin's abscess, meningitis, and osteomyelitis (Pulford et al., 2019). Although wild reptiles are considered to be the natural reservoir of *Salmonella* Panama in Latin America, in Brazil, this serotype has many other reported sources, including humans, swine, poultry, shrimp, fish, and water (Fernandes et al., 2018; Carneiro et al., 2019;

Pulford et al., 2019). Introduction of *Salmonella* Panama, among other serotypes, into the food production chain, presents a serious risk to public health and to the economy, including events such as product recalls and plant closures (Baron et al., 2017).

Food regulatory and public health agencies, such as Centers for Disease Control & Prevention (CDC), and the Food and Drug Administration (FDA), and the United States Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS), has increasingly used the whole genome sequencing (WGS) by to facilitate the detection, investigation, and control of foodborne bacterial outbreaks, and food regulatory and other activities in support of food safety (Brown et al., 2019). WGS will be used increasingly for contamination incident investigations in the animal prodution, particularly as cost continues to shrink and ease of use increases. In addition, the WGS also have a number of additional applications in the animal prodution, such as monitoring feed, identification of microbial persistence in prodution environments, and prediction of antimicrobial resistance, prediction of heavy metal and biocides resistance, and other relevant phenotypes, facilitating the improvement of sanitary management, microbial hazard control, and microbiological risk assessment (Tang et al., 2019). WGS is a high-resolution technology at the species, strain and molecular level that is predicted to become an indispensable tool in aquaculture (Bayliss et al., 2017; Bachand et al., 2020).

Thus, the present study was undertaken to investigate non-typhoidal *Salmonella* strain isolated from a sample of pond water at a fish farm for potential pathogenicity (virulence genes), antimicrobial resistance (AMR genes) and biocide and heavy metals resistance (biocides and heavy metals genes) using whole genome sequencing.

2. Material and methods

2.1. Strain isolation

Salmonella enterica. SAL78 was isolated from a sample of pond water at a fish farm of Tilapia (*Oreochromis niloticus*) in the state of Mato Grosso do Sul, Midwestern Brazil, in November 2017. The detection of *Salmonella* was based on standard methods (ISO 6579:2002/Amd.1:2007). *Salmonella* was confirmed by PCR for *inv*A gene F – GTGAAATTATCGCCACGTTCGG R – TCATCGCACCGTCAAA (Rahn et al. 1992). The serotyping used slide agglutination according to Kauffmann–White. The isolate was serotyped at the Brazilian National Reference Laboratory of Enterobacteria of the Oswaldo Cruz Foundation, Brazilian Ministry of Health.

2.2. DNA extraction

Genomic DNA was extracted using a PureLink™ Quick Gel Extraction Kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA quality and quantity were evaluated by agarose gel electrophoresis and using a Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA).

2.3 Whole genome sequence

The DNA library was prepared using a Nextera XT DNA Library Preparation Kit (Illumina Inc., Cambridge, UK), according to the manufacturer's instructions; genomic DNA was sequenced on an Illumina NextSeq® platform, using 75-bp paired-end reads. *De novo* genome assembly was performed by CLC Genomic Workbench 12 and the contigs were annotated by the NCBI Prokaryotic Genome Annotation Pipeline v.4.11 (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

2.4 *In silico* MLST

Multilocus sequence type (MLST) was predicted using MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/);

2.5 *In silico* detection of virulence genes

Virulence factors were analyzed using the databases of *Salmonella enterica* virulence gene sequences available in the Virulence Factor Database (VFDB) (http://www.mgc.ac.cn/VFs/).

2.6 *In silico* detection of resistance gene, biocides and heavy metals genes, plasmid

The *in silico* AMR gene was identified by ResFinder 3.2 (https://cge.cbs.dtu.dk/services/ResFinder/). The genes encoding resistance to biocides and heavy metals using The Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/) and BLASTX, with the BacMet2 experimentally confirmed resistance gene database v. 2.2.26 (http://bacmet.biomedicine.gu.se/blast/blast_link.cgi). The plasmids were confirmed using PlasmidFinder 2.1 (https://cge.cbs.dtu.dk//services/PlasmidFinder/).

2.7 Nucleotide sequence accession numbers

This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank.

3. Results

3.1 Whole Genome Sequencing of *Salmonella enterica*

De novo genome assembly was performed by CLC Genomic Workbench 12 and the contigs were annotated by the NCBI Prokaryotic Genome Annotation Pipeline v.4.11

(http://www.ncbi.nlm.nih.gov/genome/annotation_prok/). A total of 7,529,470 paired-end reads were produced with 113x coverage and assembled into 70 contigs. Genome size was calculated at 4,719,885 bp, with 4,525 protein-coding sequences and a G+C content of 52.2%. Overall, 41 tRNAs, 7 ncRNAs, 149 pseudogenes, and 2 CRISPR arrays were identified.

3.2 Serotyping and *In silico MLST typing*

The isolate was classified in *Salmonella enterica* serotype Panama. Based on the available data in the Center for Genomic Epidemiology *S. enterica* serotype Panama was assigned to ST2041.

3.3 *In silico* identification of virulence genes, resistance gene, biocides and heavy metals genes and plasmid

The virulence genes were identified using the Virulence Factors Database (VFDB) (http://www.mgc.ac.cn/VFs/); this evaluation resulted in the confirmation of 124 genes encoding virulence factors related to adherence, invasion, iron uptake, motility, regulation, secretion systems, and toxins are shown in Supplementary Table 1.

The result of the *in silico* detection of resistance gene showed *Salmonella enterica* serotype Panama encoded the aminoglycoside acetyl-transferase gene (*aac(6')-Iaa* gene) associated with resistance to aminoglycosides. Additionally, five efflux pumps super-families, including ATP-binding cassette (ABC), multidrug and toxic compound extrusion (MATE), major facilitator superfamily (MFS), resistance– nodulation cell–division (RND), and Small multidrug resistance (SMR) were identified. We also detected 120 genes encoding resistance to biocides [Acriflavine, Benzylkonium Chloride (BAC), Carbonylcyanide m-chlorophenyl hydrazone (CCCP), Chlorhexidine, 2-Chlorophenylhydrazine, Crystal Violet, Cyclohexane, Ethidium Bromide, Glycerol, Methylene Blue, Methyl Viologen, Pentane, p-xylene,

Phenylmercury Acetate, prenol, Proflavin, Rhodamine 6G, Sodium acetate, Sodium Dodecyl Sulfate (SDS), Sodium Deoxycholate (SDC), Sodium Glycocholate, Tetrachlorosalicylanilide (TCS), Tetraphenylphosphonium (TPP), Tributyltin (TBT), Triclosan, Hydrochloric acid (HCl)], and heavy metals [Antimony (Sb), Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Cooper (Cu), Gold (Au), Hydrogen Peroxide (H_2O_2), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Selenium (Se), Silver (Ag), Tellurium (Te), Tungsten (W), Vanadium (V), Zinco (Zn)], shown in Supplementary Table 2. Plasmid incompatibility groups IncFIB (pB171) and IncFII(S) were confirmed.

3.4 Nucleotide sequence accession numbers

This Whole Genome Shotgun sequencing project has been deposited at DDBJ/EMBL/GenBank under the accession JAAILJ000000000, BioProject ID PRJNA607083, BioSample SAMN14120753. The version described in this paper is version JAAILJ000000000.1 and data can be accessed at NCBI (https://www.ncbi.nlm.nih.gov/nuccore/JAAILJ000000000).

4. Discussion

The Brazil is making world leading tilapia (*Oreochromis niloticus*) producer, with production reaching 219 tons in 2015 (EMBRAPA, 2017). Unfortunately, the impressive development of production has been accompanied of the aquaculture rearing practices which high density stock ponds are stressful rearing environments, immunosuppressant for the fish, and the introduction of biological hazardous polluting substances, such as manure and fish feed containing protein ingredients of origin animal, has a consequence the presence of the bacteria able to cause diseases in human such as *Salmonella* (Sapkota et al., 2008; Han et al.,

2017; Martins et al., 2019). In our study was possible identified the presence of *S. enterica* serotype Panama in fish farm studied. *S.* Panama is more widely known for its ability to cause invasive disease and to colonize extraintestinal sites. For most salmonellae, extraintestinal colonization refers to bloodstream infection (Pulford et al., 2019). This serotype is among the 30 most common *Salmonella* serotypes known to cause human disease, with 1903 cases reported over the 11 -year period from 2003 to 2013 (an average of 173 cases/year), it nonetheless represents 1% of the serotypes detected, with a higher percentage isolated from blood (Parry et al., 2019). *S.* Panama continues to be isolated periodically in Latin America (Pulford et al., 2019). According to global *Salmonella* monitoring compiled by the World Health Organization between 2001 and 2007, *S. Panama* was between of the 20 most frequently serotyped human *Salmonella* isolates and the ninth most common serotype isolated from Latin America (Hendriksen et al., 2011) . In 2007, *S.* Panama was responsible for 1% of 3,439 cases of *Salmonella* infection across Argentina, Brazil, Chile, and Costa Rica (Pulford et al., 2019). In Brazil this serotype was isolated of the human in 2001-2007, from different sources, such as human, animal, and food in 2000-2009, in study realized for Hendriksen et al., 2011 and Rodrigues et al., 2010, respectly. In 2018 Carneiro et al., report the first *Salmonella* Panama case of meningitis in 4-month-old male newborn in Brazil. *S. enterica* serotypes are the leading foodborne enteric infectious agents of humans worldwide (Kumar et al., 2009). According to the World Health Organization (WHO), *Salmonella* represents the third cause of human death among food-borne diseases (WHO, 2015; Ferrari et al., 2019). The transmission of this pathogen is mainly associated with consumption the animal-based-foods, although water can also serve as vehicles (Michael and Schwarz, 2016; Ferrari et al., 2019).

Although the role of the animal-based foods in the transmission of this pathogen to humans has been demonstrated, the genetic characterization of *Salmonella* serotypes in these

products is not yet fully understood (EFSA, 2017).Thus, the emerging use of whole genome sequencing has been revolutionized the food-borne infections monitoring and public health surveillance systems (WHO, 2018; Hassena et al., 2021). The application of WGS represents an alternative method used to characterize *Salmonella* strains (virulotyping, antimicrobial resistance, biocide and heavy metals resistance and plasmid) through a single workflow more rapidly and efficiently compared to conventional molecular methods, such as Pulsed-field gel electrophoresis (PFGE), Polymerase chain reaction (PCR) which are gold standard methods for *Salmonella* molecular typing and identification of the virulence genes, antimicrobial resistance genes, respectively (WHO, 2018; Brown et al., 2019; Ribot et al., 2019; Hassena et al., 2021). Nevertheless, these molecular techniques are usually time consuming, laborious, and lack reproducibility between laboratories. Besides, they may not reflect the pathogenic potentialities of the pathogen (Hassena et al., 2021). In the present study, *Salmonella* Panama was studied through WGS.

In silico MLST revealed *Salmonella* Panama was assigned to ST2041. This Sequence type (ST) has already been reported in strains of *Salmonella* serotypes Abaetetuba, Enteritidis, Ilala, Infantis, and Poona, and recovered from a variety of samples (human, environment, aquatic animals, food) from regions and countries worldwide. To the best of our knowledge, this is the first report of this ST in the *Salmonella* serotype Panama (Enterobase, 2021).

Of particular concern is the prevalence of antimicrobial resistance genes with in NTS in fish farm. The *in silico* antibiotic resistance gene analysis showed presence of the *aac(6')-Iaa* gene. Overall, the presence of resistance genes could be explained by the unreasonable extensive use of antimicrobials for animal therapy, prophylaxis and growth promotion (Cabello et al., 2013; Hassena et al., 2021).

Regarding heavy metal resistance genes, the presence of these genetic determinants in *Salmonella* from fish farm may be due to selective pressure by antimicrobial compounds used

in aquaculture such as heavy metals and disinfectants (Cabello et al., 2013). Aquaculture and agricultural practices contribute to this world wide pollution due to diverse applications of metals in feed additives, organic and inorganic fertilizers, pesticides, and anti-fouling product, may accumulate in soil, water and sediments (Seiler and Berendonk,. 2012; Wales, Davies, 2015). Fish farmers frequently use pharmaceuticals, such as antibiotics, and metal containing products to prevent fouling, to feed and to treat fish in order to limit the spread of infections. Therefore, bacterial communities of aquacultures are strongly exposed to the combination of heavy metals and antibiotics. The exposure to both antimicrobial substances may increase the likelihood of selection and co-selection of antibiotic resistance (Seiler and Berendonk,. 2012). Co-selection of genes that confer resistance to antimicrobials, biocides, heavy metals and other chemical hazards is a potentially ecologically and clinically important phenomenon. This may occur when resistance genes to both antimicrobials and metals/biocides are co-located together in the same cell (co-resistance), or a single resistance mechanism, such as efflux pump, confers resistance to both antimicrobials and biocides/ metals (cross-resistance), leading to co-selection of bacterial strains, or mobile genetic elements (MGEs) that they carry (Cheng et al., 2019).

Genes encoding members of efflux pump super-families, including ABC, MATE, MFS, RND, and SMR were identified in this study. These families might contribute antimicrobial resistance in *Salmonella*. Of the various bacterial resistance mechanisms against antimicrobial agents, multidrug efflux pumps comprise a major cause of multiple drug resistance. These multidrug efflux pump systems reside in the biological membrane of the bacteria and actively extrude antimicrobial agents from bacterial cells (Andersen et al., 2015).

The screening of the virulence genes by the *in silico* analysis highlighted that genes encoding virulence factors related to adherence, invasion, iron uptake, motility, regulation, secretion systems, and toxins. Our data show that *S.* Panama isolated from fish farm carry

virulence genes which to play an important role in invasion, survival and adhesion. Few studies have examined the virulence genes in *S.* Panama (Carneiro et al., 2019). The presence and expression of these virulence genes could play an important role and contribute to the capacity of this serotype to cause illnesses, including invasive illnesses such as meningitis, as has already been shown with other serotypes of *Salmonella* species (Carneiro et al., 2018). Several fimbrial genes (*bcf, fim, inv, csg*) and type III secretion systems 1 and 2, implicated in cell invasion and viability of the bacteria within phagocytes that result in internalization of the bacteria which then leads to systemic spread were detected in *Salmonella* Panama. These genes are probably part of the core genes with an essential function for *Salmonella* serotype (Hassena et al., 2021).

Two typhoid-associated virulence factors genes, the cytolethal distending toxin *cdtB* gene, encoding toxins that induce apoptosis of infected cells (Fuentes et al., 2008), and the pore-forming hemolysin *clyA* gene, required for human epithelial cells invasion (Hassena et al., 2021), were identified in *Salmonella* Panama. The cytolethal distending toxin *cdtB* gene encoding is characteristic of the highly invasive *S. enterica* Typhi (Pulford et al., 2019). In *S.* Typhi, and *cdtB* requires two genes *pltA* (pertussis-like toxin A) and *pltB* (pertussis-like toxin B) to induce signs of intoxication eukaryotic cells such as cellular distension and cell cycle arrest (Mezal et al., 2014). The genes *pltA* and *pltB* also were detected in *Salmonella* Panama. In addition, the *ClyA* is an important virulence factor and a potential gene of *Salmonella* pathogenicity island 18 (SPI-18) (Fuentes et al., 2008).This gene has been shown to cause hemolysis of erythrocytes from different species (e.g. human, horse, sheep), to be cytotoxic and apoptogenic to human and murine monocytes/macro-phages, and to affect Ca^{2+} signaling in epithelial cells (von Rhein et al., 2009). These four genes were detected in *Salmonella* Panama in this study; suggest the potential virulence this isolated. The presence these virulence factors might have been horizontally transferred from *S.* Typhi to non-typhoidal

serotypes (Mezal et al., 2013, 2014), and it may contribute to a higher capacity of invasion in *Samonella* Panama. Further, was detected in *Salmonella* Panama Pathogenicity Island (SPI) composed of virulence genes such as *phoP, phoQ, sipA* and *sipB*. The genes *phoP* and *phoQ* are responsible for the control of *HilE* expression which in turn regulates the expression of SPI-1 (Monte et al., 2019). Additionally, the others virulence genes (*sipAB*)are involved in modification of lipid A (intracelular survival and ions transport), mechanisms not only involved in pathogenicity, but also in AMR (Monte et al., 2019).

The chemotaxis genes *cheA, cheB, cheR, cheW, cheY,* and c*heZ* were detected in *Salmonella* Panama. The presence this genes confer important characteristic, such as biofilm formation, cell autoaggregation and adhesion, and the colonization of hosts by pathogens (Huang et al., 2019). Thus, Chemotaxis has an important role in the infection process of pathogenic bacteria such as *Salmonella*.

Plasmids in *Salmonella* spp. have been reported to transfer antibiotics resistance and virulence traits (Carattoli, 2003; Hradecka et al., 2008). Resistance plasmids have been described to carry genes encoding virulence factors, such as bacteriocins, siderophores, cytotoxins, or adhesion factors (Martinez and Baquero, 2002). Several MDR plasmids with different Inc types have been identified in *Salmonella* (Kaldhone et al., 2019; Aljahdali et al., 2020). In this study, the *S.* Panama isolate carried IncFIB and IncFII plasmids. The IncFIB plasmids is one of the key groups of antimicrobial resistance plasmids that often carry multiple resistance determinants and virulence factors that may help bacterial pathogens improve survival capability in food animal environments and may cause infections in humans (Aljahdali et al., 2019; Kaldhone et al., 2019; Aljahdali et al., 2020). One of the key features of many IncFIB plasmids is that they often possess iron acquisition genes (e.g., sit and aerobactin operons), which can play a significant role in persistence of *Salmonell*a in the host cell, where iron is in limited supply (Khajanchi et al., 2017; Aljahdali et al., 2020). These

genes can facilitate the chelation of iron from the host during the successful infection. Additionally, IncFIB plasmids can contain antimicrobial resistance and virulence factors, which likely contribute to increase virulence of *S.* Panama during infection of host cells due to carrying iron acquisition genes. The IncFII plasmid family can replicate in many different enterobacterial species, and is clearly playing an important role in the dissemination of antimicrobial resistance genes. The plasmid of the IncFII group, carrying genes conferring multidrug-resistance has been found in *S. enterica* and enterobacterial species (Guerra et al., 2002; Feng et al., 2017). The acquisition of resistance on virulence plasmids could represent a novel tool in the bacterial evolution, implementing adaptive strategies to explore and colonise novel hosts and environments (Martinez and Baquero, 2002).

In summary, we present the first draft genome sequence of a *Salmonella* Panama ST2041 isolated from pond water at a fish farm of *Oreochromis niloticus*. Data from this genome can provide significant information with respect to the acquisition and dissemination of biocide and heavy metal resistance genes and their contributions to the food-animal-environmental interface. This information will provide important insights into the design of measures to prevent and to control the dissemination of *Salmonella* Panama in aquaculture. Moreover, the panel of virulence genes identified in this genome may confer greater understanding of the mechanisms underlying bacterial pathogenicity associated with infections in humans and other hosts.

5. Conclusions

Fish production has become a major focus of Brazilian agribusiness; However, Salmonella has been detected in aquaculture facilities, and the presence this pathogen has negative economic consequences; Multiple resistance determinants in *S*. Panama from fish farm of tilapia were identified by whole-genome sequencing. Sequence analysis further

revealed candidate genes for virulence. To the best of our knowledge, this report describes the first environment presence *S.* Panama ST2041 isolated from tilapia pond. Results suggested that tilapia pond is a potential source for contamination of food with drug-resistant *S.* Panama, which possess efflux pump genes. Our data provide the foundation for future experiments designed to delineate the pathway of pathogenesis of *S.* Panama infections. The emerging use of whole genome sequencing revolutionized in between the food-animal-environmental interface. The application of WGS represents an method to identification of microbial persistence in animal prodution environments, and prediction of antimicrobial resistance, prediction of heavy metal and biocides resistance, and other relevant phenotypes, facilitating the improvement of sanitary management in aquaculture.

Declaration Interest

None

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Supplementary Table 1. Virulence (VFPB).

Gene	Protein	Identified Virulence Factors	Identity	E-value
bcfC	outer membrane usher protein FimD [Bcf] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2645/2667 (99%)	0.0
bcfD	mannose binding protein FimH [Bcf] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	996/1008 (98%)	0.0
cdtB	cytolethal distending toxin B [Typhoid toxin] [*Salmonella enterica* subsp. enterica serovar Schwarzengrund str. CVM19633]	Toxin	783/810 (96%)	0.0
cheA	chemotaxis protein CheA [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	invasion	2002/2016 (99%)	0.0
cheB	chemotaxis-specific methylesterase [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimuriumstr. LT2]	invasion	1041/1050 (99%)	0.0
cheR	chemotaxis methyltransferase CheR [peritrichous flagella][*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	invasion	856/867 (98%)	0.0
cheW	purine-binding chemotaxis protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	invasion	499/504 (99%)	0.0
cheY	chemotaxis regulatory protein CheY [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	invasion	388/390 (99%)	0.0
cheZ	chemotaxis regulator CheZ [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	invasion	639/645 (99%)	0.0
clpV	chaperone ATPase [SCI (Salmonella centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Secretion system	2602/2629 (98%)	0.0
clyA	hemolysin E [ClyA] [*Salmonella enterica* subsp. *enterica* serovar Typhi str. CT18]	Toxin	902/918 (98%)	0.0
csgD	DNA-binding transcriptional regulator CsgD [Agf/Csg] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	649/651 (99%)	0.0
csgG	curli production assembly/transport component CsgG [Agf/Csg] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	831/834 (99%)	0.0
fimD	outer membrane usher protein SfmD [Fim] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2594/2613 (99%)	0.0
fimH	mannose binding protein FimH [Fim] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	999/1008 (99%)	0.0
flgD	flagellar basal body rod modification protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	696/699 (99%)	0.0
flgE	flagellar hook protein FlgE [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1204/1212 (99%)	0.0

flgF	flagellar basal body rod protein FlgF [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	749/756 (99%)	0.0
flgG	flagellar basal body rod protein FlgG [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	775/783 (98%)	0.0
flgH	flagellar basal body L-ring protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	696/699 (99%)	0.0
flgI	flagellar basal body P-ring protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1083/1098 (98%)	0.0
flgJ	flagellar rod assembly protein/muramidase FlgJ [peritrichousflagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	940/951 (98%)	0.0
flgk	flagellar hook-associated protein FlgK [peritrichous flagella] [*Salmonella enterica* subsp. e*nterica* serovar Typhimurium str. LT2]	Motility	1646/1662 (99%)	0.0
flgL	flagellar hook-associated protein FlgL [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	942/954 (98%)	0.0
flhA	flagellar biosynthesis protein FlhA [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	2052/2079 (98%)	0.0
flhB	flagellar biosynthesis protein FlhB [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1142/1152 (99%)	0.0
flhC	transcriptional activator FlhC [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	577/579 (99%)	0.0
flhD	transcriptional activator FlhD [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	349/351 (99%)	0.0
flhE	flagellar protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	387/393 (98%)	0.0
fliA	flagellar biosynthesis sigma factor [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	719/720 (99%)	0.0
fliB	lysine-N-methylase [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1177/1206 (97%)	0.0
fliD	flagellar capping protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1387/1404 (98%)	0.0
fliE	flagellar hook-basal body protein FliE [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	310/315 (98%)	0.0
fliF	flagellar MS-ring protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1661/1683 (98%)	0.0
fliG	flagellar motor switch protein G [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	988/996 (99%)	0.0
fliH	flagellar assembly protein H [peritrichous flagella] [*Salmonella enterica* subsp. *enteric*a serovar Typhimurium str. LT2]	Motility	698/708 (98%)	0.0

fliI	flagellum-specific ATP synthase [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1356/1371 (98%)	0.0
fliJ	flagellar biosynthesis chaperone [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	443/444 (99%)	0.0
fliK	flagellar hook-length control protein [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1204/1224 (98%)	0.0
fliL	flagellar basal body-associated protein FliL [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	463/468 (98%)	0.0
fliM	flagellar motor switch protein FliM [peritrichous flagella][*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	1002/1005 (99%)	0.0
fliN	flagellar motor switch protein FliN [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	412/414 (99%)	0.0
fliO	flagellar biosynthesis protein FliO [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	370/375 (98%)	0.0
fliP	flagellar biosynthesis protein FliP [peritrichous flagella] *Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	728/738 (98%)	0.0
fliQ	flagellar biosynthesis protein FliQ [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	267/270 (98%)	0.0
fliR	flagellar biosynthesis protein FliR [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	789/795 (99%)	0.0
fliS	flagellar protein FliS [peritrichous flagella] [*Salmonella ente*rica subsp. *enterica* serovar Typhimurium str. LT2]	Motility	407/408 (99%)	0.0
fliT	flagellar biosynthesis protein FliT [peritrichous flagella] [Salmonella enterica subsp. *enterica* serovar Typhimurium str. LT2]	Motility	365/369 (98%)	0.0
fliY	cystine transporter subunit [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	784/801 (97%)	0.0
fliZ	flagella biosynthesis protein FliZ [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	544/552 (98%)	0.0
fur	transcriptional repressor of iron-responsive genes (Fur family) (ferric uptake regulator) [Fur] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Regulation	452/453 (99%)	0.0
invA	invasion protein InvA [TTSS (SPI-1 encode)] [*Salmonella enterica* subsp. e*nterica* serovar Agona str. SL483]	Secretion system	2051/2058 (99%)	0.0
iroC	ABC transporter protein [Salmochelin] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Regulation	3586/3654 (98%)	0.0
iroC	ABC transporter protein [Salmochelin] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Iron uptake	3586/3654 (98%)	0.0

iroN	outer membrane receptor FepA [Salmochelin] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Iron uptake system	2136/2175 (98%)	0.0
mgtB	magnesium-translocating P-type ATPase [Mg2+ transport] [*Salmonella enterica* subsp. *enterica* serovarSchwarzengrund str. CVM19633]	Magnesium uptake	2713/2727 (99%)	0.0
mgtC	conserved hyopthetical protein [Mg2+ transport] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi A str. ATCC 9150]	Magnesium uptake	694/696 (99%)	0.0
misL	outer membrane autotransporter barrel domain [MisL] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Nonfimbrial adherence determinants	2846/2868 (99%)	0.0
motA	flagellar motor protein MotA [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Motility	884/888 (99%)	0.0
motB	flagellar motor protein MotB [peritrichous flagella] [*Salmonella enterica* subsp. e*nterica* serovar Typhimurium str. LT2]	Motility	910/930 (97%)	0.0
pegA	putative fimbrial subunit protein [Peg] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	534/534 (100%)	0.0
pegC	fimbrial usher protein [Peg] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2475/2487 (99%)	0.0
pegD	putative fimbrial protein [Peg] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	1006/1023 (98%)	0.0
phoP	response regulator [PhoPQ] [*Salmonella enterica* subsp.*enterica* serovar Typhimurium str. LT2]	Regulation	666/675 (98%)	0.0
phoQ	sensor protein PhoQ [PhoPQ] [*Salmonella enterica* subsp.*enterica* serovar Schwarzengrund str. CVM19633]	Regulation	1462/1464 (99%)	0.0
pipB	secreted effector protein [TTSS-2 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	866/876 (98%)	0.0
pltA	pertussis toxin, subunit 1 subfamily [Typhoid toxin] [*Salmonella enterica* subsp. *enterica* serovarSchwarzengrund str. CVM19633]	Toxin	728/729 (99%)	0.0
pltB	toxin subunit [Typhoid toxin] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi A str. ATCC 9150]	Toxin	401/414 (96%)	0.0
safB	periplasmic fimbrial chaperone protein [Saf] [*Salmonella enterica* subsp. *enterica* serovar Newport str. SL254]	Adherence	731/741 (98%)	0.0
safC	outer-membrane fimbrial usher protein [Saf] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2484/2511 (98%)	0.0
shdA	[ShdA] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	5309/5367 (98%)	0.0
sifA	secreted effector protein [TTSS-2 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	996/1011 (98%)	0.0
sifB	secreted effector protein [TTSS-2 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	939/951 (98%)	0.0

sinH	Aec1 [SinH] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2177/2193 (99%)	0.0
sipA	pathogenicity island 1 effector protein (function unknown) [TTSS-1 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Typhi str. CT18]	Secretion system	2026/2058 (98%)	0.0
sipB	cell invasion protein SipB [TTSS-1 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	1780/1782 (99%)	0.0
slrP	leucine-rich repeat protein [TTSS effectors translocated via both systems] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	2264/2268 (99%)	0.0
sopA	secreted effector protein [TTSS-1 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	2334/2349 (99%)	0.0
sopB	(sopB/sigD) inositol phosphate phosphatase SopB [TTSS-1 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	1680/1686 (99%)	0.0
sopD2	hypothetical protein [TTSS-2 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Agona str.SL483]	Secretion system	911/960 (94%)	0.0
sopE2	hypothetical protein [TTSS-1 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi B str. SPB7]	Secretion system	716/723 (99%)	0.0
ssaC	type III secretion outer membrane pore, YscC/HrcC family [TTSS (SPI-2 encode)] [*Salmonella enterica* subsp. *Enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	1485/1494 (99%)	0.0
ssaN	type III secretion system ATPase [TTSS (SPI-2 encode)] [*Salmonella enterica* subsp. *enterica* serovar Dublin str. CT_02021853]	Secretion system	1317/1332 (98%)	0.0
ssaV	secretion system apparatus protein SsaV [TTSS (SPI-2 encode) [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	2021/2046 (98%)	0.0
ssaV	hypothetical protein [TTSS (SPI-2 encode)] [*Salmonella enterica* subsp. *arizonae* serovar 62:z4,z23:-- str. RSK2980]	Secretion system	1797/1977 (90%)	0.0
sseC	Secretion system effector SseC [TTSS (SPI-2 encode)] [*Salmonella enterica* subsp. *enterica* serovar Choleraesuis str. SC-B67]	Secretion system	1430/1455 (98%)	0.0
sseJ	secreted effector protein [TTSS-2 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	1226/1227 (99%)	0.0
sseL	deubiquitinase [TTSS-2 translocated effectors] [*Salmonella enterica* subsp. *enterica* serovar Newport str. SL254]	Secretion system	987/1023 (96%)	0.0
ssrA	secretion system regulator:Sensor component [TTSS (SPI-2 encode)] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Secretion system	2724/2763 (98%)	0.0

staA	pilin chaperone ecpD2 [Sta] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	588/594 (98%)	0.0
staB	putative chaperone protein EcpD [Sta] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	737/738 (99%)	0.0
staC	putative outer membrane usher protein [Sta] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2654/2667 (99%)	0.0
staD	hypothetical protein [Sta] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi B str. SPB7]	Adherence	559/567 (98%)	0.0
staE	hypothetical protein [Sta] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi B str. SPB7]	Adherence	608/609 (99%)	0.0
staF	protein YadK [Sta] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	564/564 (100%)	0.0
staG	putative fimbrial-like adhesin protein [Sta] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	1258/1269 (99%)	0.0
stbC	outer membrane fimbrial usher protein [Stb] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2562/2562 (100%)	0.0
stbD	putative fimbrial usher [Stb] [*Salmonella enterica* subsp. e*nterica* serovar Schwarzengrund str. CVM19633]	Adherence	1326/1326 (100%)	0.0
stdB	fimbrial usher protein [Std] [*Salmonella enterica* subsp. *enterica* serovar Heidelberg str. SL476]	Adherence	2452/2490 (98%)	0.0
steA	type III secretion system effector SteA [SPI-1 encoded T3SS] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Adherence	603/633 (95%)	0.0
steC	type III secretion system effector SteC [SPI-2 encoded T3SS] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Adherence	1273/1364 (93%)	0.0
sthE	putative major fimbrial subunit [Sth] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	1076/1086 (99%)	0.0
stiA	hypothetical protein [Sti] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi B str. SPB7]	Adherence	545/552 (98%)	0.0
stiB	hypothetical protein [Sti] [*Salmonella enterica* subsp. *enterica* serovar Paratyphi B str. SPB7]	Adherence	684/684 (100%)	0.0
stiC	outer membrane usher protein FimD [Sti] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2540/2547 (99%)	0.0
stiH	putative fimbrial protein precurosr [Sti] [*Salmonella entérica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	1077/1077 (100%)	0.0
stjB	fimbrial usher protein [Stj] [*Salmonella enterica* subsp. *enterica* serovar Agona str. SL483]	Adherence	2381/2409 (98%)	0.0
tcfA	putative fimbrial protein [Tcf] [*Salmonella enterica* subsp. e*nterica* serovar Schwarzengrund str. CVM19633]	Adherence	701/711 (98%)	0.0

tcfB	CS1 type fimbrial major subunit [Tcf] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	572/576 (99%)	0.0
tcfC	TcfC protein [Tcf] [*Salmonella enterica* subsp. *enterica* serovar Schwarzengrund str. CVM19633]	Adherence	2674/2688 (99%)	0.0
tcfD	putative fimbrail protein [Tcf] [*Salmonella enterica* subsp. *enterica* serovar Heidelberg str. SL476]	Adherence	1049/1053 (99%)	0.0
flk	flagella biosynthesis regulator [peritrichous flagella] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Adherence	988/1002 (98%)	0.0
sciS/icmF-like	inner membrane protein [SCI (*Salmonella* centrosome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	3797/3870 (98%)	0.0
sciC	cytoplasmic protein [SCI (*Salmonella* centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	1862/1884 (98%)	0.0
sciI	cytoplasmic protein [SCI (*Salmonella* centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	1495/1509 (99%)	0.0
sciO	cytoplasmic protein [SCI (*Salmonella* centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	1322/1344 (98%)	0.0
sciP	hypothetical protein [SCI (*Salmonella* centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	1292/1305 (99%)	0.0
sciB	cytoplasmic protein [SCI (*Salmonella* centrisome island)][*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	983/996 (98%)	0.0
sciF	cytoplasmic protein [SCI (*Salmonell*a centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	883/892 (98%)	0.0
sciE	cytoplasmic protein [SCI (*Salmonella* centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	800/815 (98%)	0.0
sciT	cytoplasmic protein [SCI (Salmonella centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	752/789 (95%)	0.0
sciQ	inner membrane protein [SCI (Salmonella centrisome island)] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Involves in virulence	712/774 (91%)	0.0
pagN	adhesin/invasin protein PagN [Rck] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Adherence and invasion	713/720 (99%)	0.0
bapA	proline/threonine-rich protein [RatB] [*Salmonella enterica* subsp. *enterica* serovar Typhimurium str. LT2]	Secretion system	11259/11475 (98%)	0.0

Supplementary Table 2. Heavy Metal and Biocide Genes (BacMet).

Gene	BacMet ID	Accession UniProtKB	Compound	Similar to	Description	Resistance to	Code for:	Family
acrA	BAC0005	P0AE06	Acriflavine [class: Acridine], Phenol [class: Phenolic compounds], Triclosan [class: Phenolic compounds], p-xylene [class: Aromatic hydrocarbons], Cyclohexane [class: Cycloalkane], Pentane [class: Alkane]	*Escherichia coli* (strain K12)	AcrAB is a drug efflux protein with a broad substrate specificity.t can confer resistant to ampicillin, chloramphenicol as well.	biocide	Efflux	RND superfamily, MFP family
acrB	BAC0006	P31224	Acriflavine [class: Acridine], Phenol [class: Phenolic compounds], Triclosan [class: Phenolic compounds], p-xylene [class: Aromatic hydrocarbons], Cyclohexane [class: Cycloalkane], Pentane [class: Alkane]	*Escherichia coli* (strain K12)	AcrAB is a drug efflux protein with a broad substrate specificity.t can confer resistant to ampicillin, chloramphenicol as well.	biocide	Efflux	RND superfamily, AcrB/AcrD/AcrF family
acrD	BAC0563	Q8ZN77	Copper (Cu), Zinc (Zn)	*Salmonella enterica* serovar Typhimurium	Acriflavine resistance protein D; participates in the efflux of aminoglycosides. It	metal	Efflux	RND superfamily, AcrB/AcrD/AcrF family

					confers resistance to a variety of these substances. It contributes to copper and zinc resistance in Salmonella.			
acrD/yffA	BAC0008	P24177	Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Sodium Deoxycholate (SDC) [class: Acid]	*Escherichia coli* (strain K12)	Acriflavine resistance protein D; participates in the efflux of aminoglycosides.	biocide	Efflux	RND superfamily, AcrB/AcrD/AcrF family
acrE/envC	BAC0009	P24180	Acriflavine [class: Acridine], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Sodium Deoxycholate (SDC) [class: Acid], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Methyl Viologen [class: Paraquat], Ethidium Bromide [class: Phenanthridine]	*Escherichia coli* (strain K12)	Acriflavine resistance protein E; May affect specific membrane functions, such as septum formation during cell division, and cell membrane permeability. It can confer resistance to novomycin.	biocide	Efflux	RND superfamily, MFP family
acrF/envD	BAC001	P24181	Acriflavine [class: Acridine], Sodium	*Escherichia coli*	Acriflavine resistance protein F; Involved in	biocide	Efflux	RND superfamily,

	0		Dodecyl Sulfate (SDS) [class: Organo-sulfate], Sodium Deoxycholate (SDC) [class: Acid], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Methyl Viologen [class: Paraquat], Ethidium Bromide [class: Phenanthridine]	*(strain K12)*	cell envelope formation. It is produced in extremely low amounts.			AcrB/AcrD/AcrF family
acrR/ybaH	BAC0011	POACS9	Acriflavine [class: Acridine]	*Escherichia coli (strain K12)*	HTH-type potential regulator protein for the acrAB genes.	biocide	Regulator	RND superfamily, AcrB/AcrD/AcrF family
actP/yjcG	BAC0564	P32705	Sodium Glycocholate [class: Acid], Sodium acetate [class: Acetate]	*Escherichia coli (strain K12)*	Transports acetate. Also able to transport glycolate.	biocide	Membrane transporter/Enzyme	Sodium:solute symporter (SSF) family
baeR	BAC0596	D0ZNE3	Copper (Cu), Zinc (Zn), Tungsten (W), Sodium Deoxycholate (SDC) [class: Acid]	*Salmonella Typhimurium*	Member of the two-component regulatory system BaeS/BaeR. Activates BaeR by phosphorylation which then activates the mdtABCD and probably the casABCDE-ygbT-ygbF	biocide/metal	Regulator	Contains 1 response regulatory domain

					operons.			
baeR	BAC0039	P69228	Zinc (Zn), Tungsten (W), Sodium Deoxycholate (SDC) [class: Acid]	*Escherichia coli (strain K12)*	Member of the two-component regulatory system BaeS/BaeR. Activates the mdtABCD and probably the casABCDE-ygbT-ygbF operons. Overexpression of BaeR increases resistance to novobiocin and deoxycholate. BaeR upregulates the mdtABCD locus and acrD and thereby increases the resistance to deoxycholate, metals and antibiotics.	biocide/metal	Regulator	Contains 1 response regulatory domain
baeS	BAC0040	P30847	Zinc (Zn), Tungsten (W), Sodium Deoxycholate (SDC) [class: Acid]	*Escherichia coli (strain K12)*	Member of the two-component regulatory system BaeS/BaeR. Activates BaeR by phosphorylation which then activates the mdtABCD and probably the casABCDE-ygbT-ygbF operons.	biocide/metal	Regulator	Contains 1 HAMP domain, 1 histidine kinase domain.

baeS	BAC0597	D0ZNE2	Copper (Cu), Zinc (Zn), Tungsten (W), Sodium Deoxycholate (SDC) [class: Acid]	*Salmonella Typhimurium*	Member of the two-component regulatory system BaeS/BaeR. Activates BaeR by phosphorylation which then activates the mdtABCD and probably the casABCDE-ygbT-ygbF operons.	biocide/metal	Regulator	Contains 1 HAMP domain, 1 histidine kinase domain.
bcr	BAC0041	P28246	Acriflavine [class: Acridine]	*Escherichia coli (strain K12)*	Involved in sulfonamide (sulfathiazole) and bicyclomycin resistance. Probable membrane translocase	biocide	Enzyme	MFS superfamily
cepA	BAC0061	Q8RR17	Chlorhexidine [class: Biguanides]	*Klebsiella pneumoniae*	Cation-efflux pump cepA, Cation-efflux transporter that may have a role in detoxification by similarity. Associated with chlorhexidine resistance.	biocide	Efflux	Cation diffusion facilitator (CDF) transporter family (FieF subfamily)
comR/ycfQ	BAC0076	P75952	Copper (Cu)	*Escherichia coli (strain K12)*	Represses expression of BhsA/ComC by binding to its promoter region in the absence of copper.	metal	Regulator	TetR family

copA	BAC0725	Q59385	Copper (Cu)	*Escherichia coli (strain K12)*		metal	Efflux	the p-type atpase (p-atpase) superfamily
corA	BAC0641	P0A2R8	Magnesium (Mg), Cobalt (Co), Nickel (Ni), Manganese (Mn)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	Mediates both influx and efflux of magnesium ions. Can also mediate cobalt and nickel uptake, albeit only at extracellular concentrations that are toxic to the cell. Does not transport iron.	metal	Membrane Transporter	CorA metal ion transporter (MIT) family
corA	BAC0086	P0ABI4	Magnesium (Mg), Cobalt (Co), Nickel (Ni), Manganese (Mn)	*Escherichia coli (strain K12)*	Mediates influx of magnesium ions. Can also mediate cobalt and manganese uptake, albeit only at extracellular concentrations that are toxic to the cell. Does not transport iron.	metal	Membrane Transporter	CorA metal ion transporter (MIT) family
corB	BAC0643	Q9X621	Cobalt (Co), Magnesium (Mg)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	Magnesium-transporting ATPase, P-type 1; Mediates magnesium influx to the cytosol.	metal	Enzyme	Cation transport ATPase (P-type) family
corC	BAC0088	P0A2L3	Cobalt (Co), Magnesium (Mg)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	Magnesium and cobalt efflux protein CorC; Plays a role in the transport of magnesium	metal	Efflux	UPF0053 family. Contains 2 CBS domains.

					and cobalt ions			
corD	BAC0644	Q56017	Cobalt (Co), Magnesium (Mg)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	mutations in apaG/corD give a phenotype of low-level Co2+ resistance. They also decrease Mg2+ efflux but not influx via the CorA Mg2+ transport system.	metal	Unknown	Contains 1 apaG domain
cpxA	BAC0532	C4WZK5	Hydrogen Peroxide (H2O2) [class: Peroxides], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides]	*Klebsiella pneumoniae subsp. pneumoniae NTUH-K2044*	Sensor protein of stress-related two-component regulatory system of CpxAR. It has role in oxidative stress tolerance. It has a regulatory role on MDR efflux pumps. It can confer resistance to cefepime, cefotaxime, ceftazidime and chloramphenicol which possibly are the preferred substrates of the resistance determinants for e.g. efflux pumps perhaps regulated by the Cpx regulon.	biocide	Regulator	Contains 1 histidine kinase domain
cpxR	BAC0533	C4WZK6	Hydrogen Peroxide (H2O2) [class: Peroxides],	*Klebsiella pneumoniae subsp. pneumoniae NTUH-*	Response regulator of stress-related two-component regulatory	biocide	Regulator	Contains 1 response regulator domain

			Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides]	*K2044*	system. It has role in oxidative stress tolerance. It has a regulatory role on MDR efflux pumps. It can confer resistance to cefepime, cefotaxime, ceftazidime and chloramphenicol which possibly are the preferred substrates of the resistance determinants for e.g. efflux pumps perhaps regulated by the Cpx regulon.			
cueO	BAC103	P36649	Copper (Cu)	*Escherichia coli (strain K12)*	Copper efflux oxidase (blue copper oxidase); Involved in periplasmic detoxification of copper by oxidizing Cu+ to Cu2+ and thus preventing its uptake into the cytoplasm. Possesses phenoloxidase and ferroxidase activities and might be involved in the production of polyphenolic compounds and the prevention of oxidative damage in the	metal	Enzyme	Multicopper oxidase (mco)

					periplasm.			
cueP	BAC0104	Q8ZL99	Copper (Cu)	*Salmonella enterica serovar Typhimurium*	Periplasmic copper-binding protein and has been implicated in the transfer of copper ions to SodCII in the periplasm; evolved to survive in the phagosomes of macrophages in Salmonella enterica serovar Typhimurium; CueP can functionally replace the Cus complex for periplasmic copper resistance, in particular under anaerobic conditions; CueP does not confer silver or gold resistance to Salmonella	metal	Binding protein	unknown
cuiD	BAC0106	Q8ZRS2	Copper (Cu), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Salmonella enterica serovar Typhimurium*	A multi-copper oxidase gene code for Cu-inducible periplasmic protein; CuiD is required for both copper homeostasis and the copper resistance response in the presence and in the absence of oxygen;	biocide/metal	Enzyme	Multicopper oxidase (MCO) family

					Probably involved in periplasmic detoxification of copper by oxidizing Cu+ to Cu2+ and thus preventing its uptake into the cytoplasm; cuiD may be involved in oxidative stress response			
cutA	BAC0113	P69488	Copper (Cu)	*Escherichia coli (strain K12)*	Divalent-cation tolerance protein CutA; Involved in resistance toward heavy metals	metal	Binding protein	CutA family
cutC	BAC0114	P67826	Copper (Cu)	*Escherichia coli (strain K12)*	Copper homeostasis protein CutC; Involved in copper homeostasis; Located in cytoplasm	metal	Binding protein	CutC family
cutE/Int	BAC0115	P23930	Copper (Cu)	*Escherichia coli (strain K12)*	Apolipoprotein N-acyltransferas, it attaches a fatty acid to apo-nlpE (cutF)	metal	Enzyme	CN hydrolase (Apolipoprotein N-acyltransferase subfamily)
dsbA	BAC0136	P0AEG4	Cadmium (Cd), Zinc (Zn), Mercury (Hg)	*Escherichia coli (strain K12)*	Disulfide oxidoreductase; Required for disulfide bond formation in some periplasmic proteins such as PhoA or OmpA. Acts by transferring its	metal	Enzyme	Thioredoxin family. DsbA subfamily.

					disulfide bond to other proteins and is reduced in the process. DsbA is reoxidized by DsbB. It is required for pilus biogenesis			
dsbB	BAC0137	P0A6M2	Cadmium (Cd), Mercury (Hg)	*Escherichia coli (strain K12)*	Disulfide oxidoreductase; The dsbB gene codes for a transmembrane protein that is responsible for reoxidizing the periplasmic dsbA-encoded disulfide oxidoreductase; Required for disulfide bond formation in some periplasmic proteins such as PhoA or OmpA	metal	Enzyme	DsbB family
dsbC	BAC0138	P0AEG6	Copper (Cu)	*Escherichia coli (strain K12)*	Disulfide isomerase; DsbC rearranges incorrect disulfide bonds formed by dsbA during oxidative protein folding under non-stress conditions. DsbC, a periplasmic thiol-disulfide oxidoreductase, appears to function as a disulfide isomerase	metal	Enzyme	Thioredoxin family. DsbC subfamily

					both in vitro and in vivo. In vitro, DsbC has been shown to rearrange non-native disulfides in well studied isomerization substrates such as BPTI and RNase A. In vivo, DsbC is required for full activity of a handful of proteins containing at least one non-consecutive disulfide			
emrA	BAC0147	P27303	Phenylmercury Acetate [class: Organo-mercury], 2-Chlorophenylhydrazine [class: Hydrazine], Carbonylcyanide m-chlorophenyl hydrazone (CCCP) [class: Hydrazone], Tetrachlorosalicylanilide (TCS) [class: Salicylanilide]	*Escherichia coli (strain K12)*	The emr locus confers resistance to substances of high hydrophobicity. EmrA probably participate in a transport system to extrude toxins and drugs from the cell.	biocide	Efflux	Membrane Fusion Protein (MFP)
emrB	BAC0148	P0AEJ0	Phenylmercury Acetate [class: Organo-mercury], 2-Chlorophenylhydrazine [class: Hydrazine], Carbonylcyanide m-	*Escherichia coli (strain K12)*	Multidrug resistance protein B, Located in cell inner membrane, Translocase that confers resistance to substances of high	biocide	Enzyme	Major facilitator superfamily (MFS)-EmrB family

			chlorophenyl hydrazone (CCCP) [class: Hydrazone]		hydrophobicity			
emrD	BAC0149	P31442	Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate]	*Escherichia coli (strain K12)*	Multidrug resistance pump that participates in a low energy shock adaptative response	biocide	Efflux	MFS superfamily
emrR	BAC0559	P0ACR9	Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) [class: Hydrazone]	*Escherichia coli (strain K12)*	Negative regulator of the multidrug operon emrAB.	biocide	Regulator	MarR family
fabl	BAC0156	P0AEK4	Triclosan [class: Phenolic compounds]	*Escherichia coli (strain K12)*	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI; Catalyzes the reduction of a carbon-carbon double bond in an enoyl moiety that is covalently linked to an acyl carrier protein (ACP). Involved in the elongation cycle of fatty acid which are used in the lipid metabolism and in the biotin biosynthesis; enoyl-ACP reductase is the FabI protein of	biocide	Enzyme	short-chain dehydrogenases/reductases (SDR) family.

					Escherichia coli that is the target of the antibacterial compound, triclosan; some Gram-positive bacteria are naturally resistant to triclosan due to the presence of the triclosan-resistant enoyl-ACP reductase isoforms, FabK, FabV and FabL.			
fetB/ybbM	BAC0166	P77307	Iron (Fe), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli (strain K12)*	FetB/YbbM is a membrane protein. FetAB allows for increased resistance to oxidative stress in the presence of iron	biocide/metal	Membrane protein	ABC transporter superfamily
fieF/yiip	BAC0167	P69380	Iron (Fe), Zinc (Zn), Cobalt (Co), Cadmium (Cd), Nickel (Ni)	*Escherichia coli (strain K12)*	Iron-efflux transporter responsible for iron detoxification. Also able to transport Zn2+ in a proton-dependent manner. The CDF protein, FieF, is mainly a ferrous iron detoxifying protein but also mediated some resistance against other divalent metal cations such as Zn(II), Co(II), Cd(II), and Ni(II) in W.	metal	Efflux	Cation diffusion facilitator (CDF) transporter family

					metallidurans or Escherichia coli.			
gesA	BAC0178	Q8ZRG8	Gold (Au), Methylene Blue [class: Thiazinium], Crystal Violet [class: Triarylmethane]	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	The gold (Au2+) resistance efflux pump, GesABC (induced by GolS in the presence of Au2+; also mediates drug resistance when induced by Au2+. Also exports a variety of organic chemicals including chloramphenicol.	biocide/metal	Efflux	RND superfamily, MFP family
gesB	BAC0179	Q8ZRG9	Gold (Au), Methylene Blue [class: Thiazinium], Crystal Violet [class: Triarylmethane]	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	The gold (Au2+) resistance efflux pump, GesABC (induced by GolS in the presence of Au2+; also mediates drug resistance when induced by Au2+. Also exports a variety of organic chemicals including chloramphenicol.	biocide/metal	Efflux	RND superfamily
gesC	BAC0180	Q8ZRH0	Gold (Au), Methylene Blue [class: Thiazinium], Crystal Violet [class: Triarylmethane]	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	The gold (Au2+) resistance efflux pump, GesABC (induced by GolS in the presence of Au2+; also mediates drug resistance when induced by Au2+. Also	biocide/metal	Efflux	RND Superfamily, OMP family

					exports a variety of organic chemicals including chloramphenicol.			
glpF	BAC0181	P0AER0	Antimony (Sb), Arsenic (As), Glycerol [class: Alcohol]	*Escherichia coli (strain K12)*	Glycerol uptake facilitator protein GlpF; Transporter of glycerol across the cytoplasmic membrane, with limited permeability to water and small uncharged compounds such as polyols	biocide/metal	Channel	Major Intrinsic protein (MIP) superfamily. MIP/aquaporin family
golS	BAC0182	Q8ZRG6	Gold (Au)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	merR-type regulator; GolS directly controls the expression of golT and golB; Each component of the GolS regulon, i.e. the P-type ATPase GolT; and the metal-binding polypeptide GolB, are required for full gold-tolerance	metal	Regulator	Contains 1 HTH merR-type DNA-binding domain
golT	BAC0183	Q8ZRG7	Copper (Cu), Gold (Au)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	Cation transporting P-type ATPase; belongs to the gold-responsive, gol regulon; In the absence of CopA, GolS becomes responsive to copper, inducing the	metal	Enzyme	Cation transport ATPase (P-type) family

					expression of golT, which functionally substitutes CopA for cytosolic copper export			
ibpA	BAC0194	P0C054	Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli (strain K12)*	Small heat shock protein IbpA; Associates with aggregated proteins, together with IbpB, to stabilize and protect them from irreversible denaturation and extensive proteolysis during heat shock and oxidative stress; Its activity is ATP-independent	biocide	Heat shock protein	Small heat shock protein (HSP20)
ibpB	BAC0195	P0C058	Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli (strain K12)*	Small heat shock protein IbpA; Associates with aggregated proteins, together with IbpA, to stabilize and protect them from irreversible denaturation and extensive proteolysis during heat shock and oxidative stress. Its activity is ATP-independent.	biocide	Heat shock protein	Small heat shock protein (HSP20)

icIR	BAC0196	P16528	Sodium acetate [class: Acetate]	*Escherichia coli (strain K12)*	Acetate operon repressor; Regulation of the glyoxylate bypass operon (aceBAK), which encodes isocitrate lyase, malate synthase as well as isocitrate dehydrogenase kinase/phosphorylase. Glyoxylate disrupts the interaction with the promoter by favoring the inactive dimeric form. Pyruvate enhances promoter binding by stabilizing the tetrameric form.	biocide	Regulator	Contains 1 HTH iclR-type DNA-binding domain, 1 iclR-ED (iclR effector binding) domain
kdeA	BAC0491	A6T6T9	Acriflavine [class: Acridine], Ethidium Bromide [class: Phenanthridine], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate]	*Klebsiella pneumoniae (strain MGH 78578)*	It can confer resistance to antibiotics such as chloramphenicol, Kanamycin, norfloxacin, puromycin, daunomycin etc.	biocide	Efflux	MFS superfamily
kmrA	BAC0493	C4X8X9	Acriflavine [class: Acridine], 4,6-diamidino-2-	*Klebsiella pneumoniae*	It can confer resistance to Antibiotics such as kanamycin,	biocide	Efflux	MFS superfamily

			phenylindole (DAPI) [class: Diamindine], Hoechst 33342 [class: Bisbenzimide], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Methyl Viologen [class: Paraquat], Ethidium Bromide [class: Phenanthridine], Cetrimide (CTM) [class: Quaternary Ammonium Compounds (QACs)]		gentamycin, erythromycin etc.			
kpnF	BAC0477	C4X7Z4	Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Sodium Deoxycholate (SDC) [class: Acid], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides], Triclosan [class: Phenolic compounds], Methyl Viologen [class: Paraquat], Hydrogen Peroxide (H2O2) [class: Peroxides]	*Klebsiella pneumoniae NTUH-K2044*	KpnEF is a EbrAB homolog. kpnEF is involved in oxidative stress tolerance, nitrosative stress tolerance, heat stress tolerance, osmotic stress toerance etc. in Klebsiella pneumoniae. It can confer resistance to antibiotics such as cefepime, ceftriaxone, colistin, erythromycin, rifamycin, tetracycline and streptomycin	biocide	Efflux	Small multidrug resistance (SMR) family

kpnO	BAC0529	C4XBC3	Hydrogen Peroxide (H2O2) [class: Peroxides], Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) [class: Hydrazone], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides], Sodium Nitrite (NaNo2) [class: Nitrites]	*Klebsiella pneumoniae NTUH-K2044*	KpnO codes for a outer membrane porin. kpnO mediates antibiotic resistance via efflux mechanism and contributes towards disinfectant tolerance. It has a role in oxidative stress tolerance and nitrostative stress tolernace. KpnO also contributes to growth and gastrointestinal stress tolerance in K. pneumoniae.	biocide	Porin	RND superfamily, OMP family
marA	BAC0560	P0ACH5	Cyclohexane [class: Cycloalkane], Diphenyl Ether [class: Phenyl], n-hexane [class: Alkane]	*Escherichia coli (strain K12)*	A transcriptional activator (MarA) of genes involved in the multiple antibiotic resistance (Mar) phenotype. It can also activate genes such as sodA, zwf and micF. Organic solvent tolerance and antibiotic resistance increased by overexpression of marA in Escherichia coli. High copy number of MarA and MarR also increases the tolerance level of	biocide	Regulator	Contains 1 HTH araC/xylS-type DNA-binding domain

					bacteria to organic solvents.			
mdfA/cmr	BAC0208	P0AEY8	Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Ethidium Bromide [class: Phenanthridine], Acriflavine [class: Acridine], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Sodium Hydroxide (NaOH) [class: Base], Rhodamine 6G [class: Xanthene]	*Only gram -ve, E. coli (Strain K12)*	Multidrug transporter MdfA, Efflux pump driven by the proton motive force. Confers resistance to a broad spectrum of chemically unrelated drugs. Confers resistance to a diverse group of cationic or zwitterionic lipophilic compounds such as ethidium bromide, tetraphenylphosphonium, rhodamine, daunomycin, benzylkonium, rifampicin, tetracycline, puromycin, and to chemically unrelated, clinically important antibiotics such as chloramphenicol, erythromycin, and certain aminoglycosides and fluoroquinolones. OverExpression results in isopropyl-beta-D-thiogalactopyranoside	biocide	Efflux	Major facilitator Superfamily (MdfA family)

					(IPTG) exclusion and spectinomycin sensitivity. Transport of neutral substrates is electrogenic, whereas transport of cationic substrates is electroneutral. In addition to its role in multidrug resistance, confers extreme alkaline pH resistance, allowing the growth under conditions that are close to those used normally by alkaliphiles. This activity requires Na+ or K+; also known as Chloramphenicol resistance pump Cmr			
mdtA	BAC0645	D0ZND8	Zinc (Zn)	*Salmonella enterica serovar Typhimurium*	Multidrug resistance protein MdtA. Part of a tripartite efflux system composed of MdtA, MdtB and MdtC. MdtB forms a heteromultimer with MdtC. MdtABC multidrug efflux systems has a role in metal resistance.	metal	Efflux	RND superfamily, MFP family

mdtA/yegM	BAC0210	P76397	Sodium Deoxycholate (SDC) [class: Acid]	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtA; The MdtABC tripartite complex confers resistance against novobiocin and deoxycholate; MdtABC requires TolC for its function	biocide	Efflux	RND superfamily, MFP family
mdtB	BAC0646	D0ZND9	Zinc (Zn)	*Salmonella enterica serovar Typhimurium*	Multidrug resistance protein MdtB. Part of a tripartite efflux system composed of MdtA, MdtB and MdtC. MdtB forms a heteromultimer with MdtC. MdtABC multidrug efflux systems has a role in metal resistance.	metal	Efflux	RND superfamily, AcrB/AcrD/AcrF family
mdtB/yegN	BAC0211	P76398	Sodium Deoxycholate (SDC) [class: Acid], Hydrochloric acid (HCl) [class: Acid]	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtB; The MdtABC tripartite complex confers resistance against novobiocin and deoxycholate. MdtABC requires TolC for its function.	biocide	Efflux	RND superfamily, AcrB/AcrD/AcrF family
mdtC	BAC0647	D0ZNE0	Zinc (Zn)	*Salmonella enterica serovar*	Multidrug resistance protein MdtC. Part of a tripartite efflux system	metal	Efflux	RND superfamily, AcrB/AcrD/AcrF family

				Typhimurium	composed of MdtA, MdtB and MdtC. MdtB forms a heteromultimer with MdtC. MdtABC multidrug efflux systems has a role in metal resistance.			
mdtC/yegO	BAC0212	P76399	Sodium Deoxycholate (SDC) [class: Acid]	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtC; The MdtABC tripartite complex confers resistance against novobiocin and deoxycholate. MdABC requires TolC for its function.	biocide	Efflux	RND superfamily, AcrB/AcrD/AcrF family
mdtE/yhiU	BAC0213	P37636	Crystal Violet [class: Triarylmethane], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Sodium Deoxycholate (SDC) [class: Acid], Rhodamine 6G [class: Xanthene], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtE; Part of a multidrug resistance efflux system (mdtEF-TolC) that confers resistance to compounds such as rhodamine 6G, erythromycin, doxorubicin, ethidium bromide, TPP, SDS, deoxycholate, crystal violet and benzylkonium. Part of a tripartite efflux system composed of	biocide	Efflux	RND superfamily, MFP family

			Compounds (QACs)]		MdtE, MdtF and TolC. It has a role in biofilm formation of E. coli K12.			
mdtF/yhiV	BAC0214	P37637	Crystal Violet [class: Triarylmethane], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Sodium Deoxycholate (SDC) [class: Acid], Rhodamine 6G [class: Xanthene], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)]	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtF; Part of a multidrug resistance efflux system that confers resistance to compounds such as rhodamine 6G, erythromycin, doxorubicin, ethidium bromide, TPP, SDS, deoxycholate, crystal violet and benzylkonium. Part of a tripartite efflux system composed of MdtE, MdtF and TolC.	biocide	Efflux	RND superfamily, AcrB/AcrD/AcrF family
mdtG/yceE	BAC0215	P25744	Sodium Deoxycholate (SDC) [class: Acid]	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtG; Confers resistance to fosfomycin and deoxycholate	biocide	Membrane Transporter	MFS superfamily, DHA1 family, MdtG subfamily
mdtK/ydhE	BAC0218	P37340	Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Sodium Deoxycholate	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtK; It functions as a Na+/drug antiporter. Confers resistance to	biocide	Efflux	MATE family, MdtK subfamily

			(SDC) [class: Acid], Ethidium Bromide [class: Phenanthridine], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Acriflavine [class: Acridine]		many drugs such as fluoroquinolones (norfloxacin, ciprofloxacin, enoxacin), tetraphenylphosphonium ion (TPP), deoxycholate, doxorubicin, trimethoprim, chloramphenicol, fosfomycin, Acriflavine, ethidium bromide and benzylkonium			
mdtl/ydgE	BAC0216	P69210	Sodium Deoxycholate (SDC) [class: Acid], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Spermidine [class: Polyamines]	*Escherichia coli (strain K12)*	Spermidine export protein MdtI; Catalyzes the excretion of spermidine. Can also confer resistance to deoxycholate and SDS	biocide	Enzyme	SMR superfamily
mdtM/yjiO	BAC0219	P39386	Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Ethidium Bromide [class: Phenanthridine], Acriflavine [class: Acridine], Tetraphenylarsonium (TPA) [class: Quaternary Ammonium	*Escherichia coli (strain K12)*	Multidrug resistance protein MdtM; Confers resistance to Acriflavine, chloramphenicol, norfloxacin, ethidium bromide and TPP, tetraphenylarsonium chloride ; part of a tripartite efflux system composed of MdtN,	biocide	Efflux	MFS superfamily

			Compounds (QACs)]		MdtO and MdtP			
mgtA	BAC0642	P36640	Cobalt (Co), Magnesium (Mg)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	Mediates magnesium influx to the cytosol.	metal	Enzyme	Cation transport ATPase (P-type) family
mgtA	BAC0087	P0ABB8	Cobalt (Co), Magnesium (Mg)	*Escherichia coli (strain K12)*	Magnesium-transporting ATPase, P-type 1; Mediates magnesium influx to the cytosol.	metal	Enzyme	Cation transport ATPase (P-type) family
mntH/yfeP	BAC0251	P0A769	Manganese (Mn), Iron (Fe), Cadmium (Cd), Cobalt (Co), Zinc (Zn)	*Escherichia coli (strain K12)*	Divalent metal cation transporter MntH; H+-stimulated, divalent metal cation uptake system. Involved in manganese and iron uptake. Can also transport cadmium, cobalt, zinc and to a lesser extent nickel and copper. Involved in response to reactive oxygen.	metal	Membrane Transporter	NRAMP family
mntP/yebN	BAC0252	P76264	Manganese (Mn), Magnesium (Mg), Methyl Viologen [class: Paraquat], Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli K-12*	MntP; Functions as a manganese efflux pump	biocide/metal	Efflux	MntP family

mntR	BAC0253	P0A9F1	Manganese (Mn), Magnesium (Mg)	*Escherichia coli*	Manganese transport regulator mntR; In the presence of manganese, represses expression of mntH and mntS. Up-regulates expression of mntP	metal	Regulator	Contains 1 HTH dtxR-type DNA-binding domain
modA	BAC0609	P37329	Tungsten (W), Molybdenum (Mo)	*Escherichia coli (strain K12)*	Molybdate-binding periplasmic protein. Involved in the transport of molybdenum into the cell. Binds molybdate with high specificity and affinity.	metal	Binding protein	ABC superfamily, bacterial solute-binding protein ModA family.
modB	BAC0610	P0AF01	Tungsten (W), Molybdenum (Mo)	*Escherichia coli (strain K12)*	Molybdenum transport system permease protein ModB. Part of the binding-protein-dependent transport system for molybdenum; probably responsible for the translocation of the substrate across the membrane.	metal	Enzyme	ABC superfamily, binding-protein-dependent transport system permease family. CysTW subfamily
modC	BAC0611	P09833	Tungsten (W), Molybdenum (Mo)	*Escherichia coli (strain K12)*	Molybdenum import ATP-binding protein ModC. Part of the ABC transporter complex ModABC involved in	metal	Enzyme	ABC transporter superfamily. Molybdate importer family

					molybdenum import. Responsible for energy coupling to the transport system.			
modE	BAC0608	P0A9G8	Tungsten (W), Molybdenum (Mo)	*Escherichia coli (strain K12)*	Transcriptional regulator ModE. The ModE-Mo complex acts as a repressor of the modABC operon, involved in the transport of molybdate. It controls tungstate and molybdate homeostasis in bacteria.	metal	Regulator	ModE family
nfsA	BAC0540	P17117	Chromium (Cr)	*Escherichia coli (strain K12)*	A major oxygen-insensitive nitroreductase of Escherichia coli, is a flavoprotein that is able to reduce chromate to less soluble and less toxic Cr(III). Reduction of nitroaromatic compounds using NADH. Reduces nitrofurazone by a ping-pong bi-bi mechanism possibly to generate a two-electron transfer product. Major component of the	metal	Enzyme	Flavin oxidoreductase frp family

					oxygen-insensitive nitroreductase activity in E.coli.			
nikR	BAC0275	P37617	Nickel (Ni)	*Escherichia coli (strain K12)*	Nickel-responsive regulator nikR; Transcriptional repressor of the nikABCDE operon. Is active in the presence of excessive concentrations of intracellular nickel.	metal	Regulator	Transcriptional regulatory CopG/NikR family
ompD/nmpC	BAC0290	P37592	Methyl Viologen [class: Paraquat]	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	Outer membrane porin protein OmpD; Forms pores that allow passive diffusion of small molecules across the outer membrane	biocide	Porin	Gram-negative porin family
ostA/IptD	BAC0296	P3154	n-hexane [class: Alkane]	*Escherichia coli (strain K12)*	Organic solvent tolerance protein ostA/lptD; Together with LptE, is involved in the assembly of LPS in the outer leaflet of the outer membrane. Determines N-hexane tolerance and is involved in outer membrane permeability. Essential	biocide	Porin	LptD family

					for envelope biogenesis			
oxyRkp	BAC0536	C4WZN6	Hydrogen Peroxide (H2O2) [class: Peroxides], Sodium Deoxycholate (SDC) [class: Acid], Acriflavine [class: Acridine], Rhodamine 6G [class: Xanthene], Ethidium Bromide [class: Phenanthridine], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate]	*Klebsiella pneumoniae NTUH-K2044*	Activator of hydrogen peroxide-inducible genes. It has contributory role towards varied stress tolerance in K. pneumoniae.	biocide	Regulator	LysR-family
phoB	BAC0530	C4X6T6	Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides]	*Klebsiella pneumoniae NTUH-K2044*	Part of the two-component regulatory system PhoBR. It regulates kpnO in K. pneumoniae. It has a role in role in capsule production and bacterial growth in K. pneumoniae. It has no role in oxidative stress tolerance and nitrostative stress tolernace, but in gastrointestinal like challenges.	biocide	Regulator	Contains 1 histidine kinase domain
pitA	BAC031	P0AFJ7	Zinc (Zn), Tellurium	*Escherichia coli*	Low-affinity inorganic phosphate transporter	metal	Porter	Inorganic-Phospahte Transporter (PiT) family,

	2		(Te)	*(strain K12)*	1, PitA; Can also transport arsenate			Pit subfamily
pmrG	BAC048 6	Q8ZNF4	Iron (Fe)	*Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720)*	It is a phodphatase and catalyzes the dephosphorylation of heptose(II) of the outer membrane lipopolysaccharide core and localizes to the periplasm. Required for iron(III)+ resistance.	metal	Enzyme	Phosphoglycerate mutase family. Ais subfamily
pstA	BAC031 5	P07654	Arsenic (As)	*Escherichia coli (strain K12)*	An integral membrane protein. Part of the binding-protein-dependent transport system for phosphate; probably responsible for the translocation of the substrate across the membrane.	metal	Enzyme	binding-protein-dependent transport system permease family. CysTW subfamily
pstB	BAC031 6	P0AAH0	Arsenic (As)	*Escherichia coli (strain K12)*	Phosphate transporting ATPase; Part of the ABC transporter complex PstSACB involved in phosphate import. Responsible for energy coupling to the transport system.	metal	Enzyme	ABC transporter superfamily. Phospahte importer family
pstC	BAC031	P0AGH8	Arsenic (As)	*Escherichia coli*	An integral membrane permease protein; Part	metal	Enzyme	Binding-protein-dependent transport

	7			*(strain K12)*	of the binding-protein-dependent transport system for phosphate; probably responsible for the translocation of the substrate across the membrane.			system permease family. CysTW subfamily
pstS	BAC0318	P0AG82	Arsenic (As)	*Escherichia coli (strain K12)*	A periplasmic phosphate binding protein; Part of the ABC transporter complex PstSACB involved in phosphate import.	metal	Binding protein	PstS family
rcnR/yohL	BAC0332	P64530	Cobalt (Co), Nickel (Ni), Iron (Fe)	*Escherichia coli (strain K12)*	Repressor of rcnA, rcnR. Acts by binding specifically to the rcnA promoter in the absence of nickel and cobalt. In the presence of one of these metals, it has a weaker affinity for rcnA promoter. Can repress the rcnB expression as well.	metal	Regulator	FrmR/RcnR family
robA	BAC0334	P0AC10	Silver (Ag), Mercury (Hg), Cadmium (Cd), Cyclohexane [class: Cycloalkane], Pentane [class: Alkane], n-hexane [class: Alkane],	*Escherichia coli O157:H7*	Right origin-binding protein RobA; Organic solvent tolerance by robA is dependent on soxRS; Rob acts as a transcriptional	biocide/metal	Binding protein	1 HTH araC/xylS-type DNA-binding domain

			Diphenyl Ether [class: Phenyl]		activator. It can confer resistance to antibiotics such as tetracycline, chloramphenicol, phosphomycin, kanamycin, novomycin etc.			
rpoS	BAC0335	P35540	Hydrochloric acid (HCl) [class: Acid], Sodium hydroxide (NaOH) [class: Base]	*Escherichia coli, Shigella flexneri*	RNA polymerase sigma factor RpoS; Sigma factors are initiation factors that promote the attachment of RNA polymerase to specific initiation sites and are then released; RpoS is required for optimal maintenance of pH homeostasis of stationary phase cells exposed to extreme acid.	biocide	Regulator	sigma-70 factor family
sdeX	BAC0338	Q7WSD6	Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides], Triclosan [class: Phenolic compounds], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Acriflavine [class:	*Only gram -ve, Serratia marcescens*	Multidrug efflux pump SdeX	biocide	Efflux	RND superfmaily

			Acridine], Rhodamine 6G [class: Xanthene]					
sdeY	BAC0339	Q7WSD5	Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Chlorhexidine [class: Biguanides], Triclosan [class: Phenolic compounds], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Acriflavine [class: Acridine], Rhodamine 6G [class: Xanthene]	*Only gram -ve, Serratia marcescens*	Multidrug efflux pump SdeY	biocide	Efflux	RND superfamily, MFP family
sitA	BAC0349	Q9XCS2	Manganese (Mn), Iron (Fe), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli O45:K1 (strain S88 / ExPEC)*	Iron/Mn transport protein, periplasmic-binding protein SitA; sitA gene encodes a periplasmic binding protein	biocide/metal	Binding protein	ABC superfamily (bacterial solute-binding protein 9 family)
sitB	BAC0350	Q9XCS1	Manganese (Mn), Iron (Fe), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli (strain SMS-3-5 / SECEC)*	sitB encodes an ATP-binding protein	biocide/metal	Binding protein	ABC superfamily
sitC	BAC0351	Q9XCS0	Manganese (Mn), Iron (Fe), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli (strain SMS-3-5 / SECEC)*	sitC encodes a permease (integral membrane protein); part of the sitABCD	biocide/metal	Enzyme	ABC superfamily (ABC-3 integral membrane protein family)

					transport system			
sitD	BAC0352	Q9XCR9	Manganese (Mn), Iron (Fe), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli (strain SMS-3-5 / SECEC*	sitD encode a permease (integral membrane protein); part of the sitABCD transport system	biocide/metal	Enzyme	ABC superfamily (ABC-3 integral membrane protein family)
smdA	BAC0353	A7VN01	4,6-diamidino-2-phenylindole (DAPI) [class: Diamidine], Hoechst 33342 [class: Bisbenzimide]	*Serratia marcescens*	Multidrug efflux pump SmdA	biocide	Efflux	ABC superfamily
smvA/emrB	BAC0367	D0ZWQ3	Methyl Viologen [class: Paraquat], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Hoechst 33342 [class: Bisbenzimide], Ethidium Bromide [class: Phenanthridine], Cetrimide (CTM) [class: Quaternary Ammonium Compounds (QACs)], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Acriflavine [class: Acridine]	*Salmonella typhimurium*	Methyl viologen resistance protein SmvA, Major efflux pump for Acriflavine and other quaternary ammonium compounds (QACs). Also required for resistance to methyl viologen, Mutants show increased sensitivity to methyl viologen and Acriflavine.	biocide	Efflux	Major facilitator superfamily (MFS), TCR/tet family

sodA	BAC0368	P00448	Selenium (Se), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli K12*	Mn-dependent superoxide dismutase; Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems.	biocide	Enzyme	Iron/manganese superoxide dismutase family
sodB	BAC0706	P53641	Hydrogen Peroxide (H2O2) [class: Peroxides]	*Pseudomonas aeruginosa*	Fe-dependent superoxide dismutase; Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems.	biocide	Enzyme	Iron/manganese superoxide dismutase family
sodB	BAC0707	P0AGD3	Selenium (Se), Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli K12*	Fe-dependent superoxide dismutase. Destroys superoxide anion radicals which are normally produced within the cells and which are toxic to biological systems.	biocide	Enzyme	Iron/manganese superoxide dismutase family
soxR	BAC0370	P0ACS2	Methyl Viologen [class: Paraquat]	*Escherichia coli (strain K12)*	Redox-sensitive transcriptional activator SoxR; Activates the transcription of the soxS gene which itself controls the superoxide response regulon. SoxR	biocide	Regulator	Contains 1 HTH merR-type DNA-binding domain

					contains a 2Fe-2S iron-sulfur cluster that may act as a redox sensor system that recognizes superoxide. The variable redox state of the Fe-S cluster is employed in vivo to modulate the transcriptional activity of SoxR in response to specific types of oxidative stress. Upon reduction of 2Fe-2S cluster, SoxR reversibly loses its transcriptional activity, but retains its DNA binding affinity.			
sugE	BAC0378	P69937	Cetrimide (CTM) [class: Quaternary Ammonium Compounds (QACs)], Cetylpyridinium Chloride (CPC) [class: Quaternary Ammonium Compounds (QACs)], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium	*only gram -ve, Escherichia coli*	Quaternary ammonium compound-resistance protein sugE, Quaternary ammonium compound efflux pump. Confers resistance to cetylpyridinium, cetyldimethylethyl ammonium and cetrimide cations; Unlike the other members of the SMR family, SugE exhibits a	biocide	Efflux	Small multidrug resistance (SMR) family (SugE subfamily)

			Compounds (QACs)], Ethidium Bromide [class: Phenanthridine], and Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate]		narrow specificity for a very specific class of compounds.			
tehA	BAC384	P25396	Tellurium (Te), Proflavin [class: Acridine], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Ethidium Bromide [class: Phenanthridine], Crystal Violet [class: Triarylmethane], Dequalinium [class: Quaternary Ammonium Compounds (QACs)], Methyl Viologen [class: Paraquat]	*Escherichia coli (strain K12)*	ellurite resistance protein TehA; The gene encodes an integral membrane protein. Responsible for potassium tellurite resistance when present in high copy number. Ion channel involved in potassium tellurite resistance, otherwise phenotypically silent	biocide/metal	Membrane Transporter	Tellurite-resistance/dicarboxylate transporter (TDT) family
tehB	BAC385	P25397	Tellurium (Te), Proflavin [class: Acridine]	*Escherichia coli (strain K12)*	S-adenosyl-L-methionine dependent methyltransferase tehB that catalyzes the methylation of tellurite and is responsible for tellurite resistance when present in high copy number. Can also methylate selenite and	biocide/metal	Enzyme	TehB family

					selenium dioxide. Is thus able to detoxify different chalcogens. Can not methylate arsenic compounds			
tolC	BAC0393	P02930	Phenol [class: Phenolic compounds], Triclosan [class: Phenolic compounds], Sodium Deoxycholate (SDC) [class: Acid], Sodium Cholate [class: Acid], Sodium Taurodeoxycholate [class: Acid], Sodium Dodecyl Sulfate (SDS) [class: Organo-sulfate], Proflavin [class: Acridine], Acriflavine [class: Acridine], Ethidium Bromide [class: Phenanthridine], Tetraphenylphosphonium (TPP) [class: Quaternary Ammonium Compounds (QACs)], Rhodamine 6G [class: Xanthene], Tetraphenylarsonium (TPA) [class: Quaternary Ammonium Compounds (QACs)], Cetrimide (CTM) [class:	*Escherichia coli (strain K12)*	Required for proper expression of outer membrane protein genes such as ompF, nmpC, protein 2, hemolysin, colicin V, or colicin E1. May be specialized for signal sequence independent, extracellular secretion in Gram-negative bacteria. Required for acrAB, mar efflux pumps to act as multidrug efflux pump.	biocide	Efflux	RND Superfamily, OMP family

			Quaternary Ammonium Compounds (QACs)], Dequalinium [class: Quaternary Ammonium Compounds (QACs)], Benzylkonium Chloride (BAC) [class: Quaternary Ammonium Compounds (QACs)], Plumbagin [class: Naphthoquinone]					
yddg/emrE	BAC0435	D0ZXP9	Methyl Viologen [class: Paraquat]	*Salmonella typhimurium (strain 14028s/SGSC 2262)*	Methyl viologen resistance protein YddG, Probable efflux pump. Required for resistance to methyl viologen. May function with OmpD porin	biocide	Efflux	Drug/metabolite transporter (DMT) superfamily; Aromatic amino acid/Paraquat Exporter (ArAA/P-E) family
ygiW	BAC0445	P0ADU5	Cadmium (Cd), Tributyltin (TBT) [class: Organo-tin], Hydrogen Peroxide (H2O2) [class: Peroxides]	*Escherichia coli*	Protein YgiW; located in periplasm; responsible for tributyltin (TBT) resistance; response to hydrogen peroxide (H2O2), cadmium ions ; has a role in biofim formation	biocide/metal	Unknown	Bacterial OB fold (BOF) protein family
yhcN	BAC0446	P64614	Cadmium (Cd), Hydrogen Peroxide (H2O2) [class: Peroxides],	*Escherichia coli (strain K12)*	Outer membrane protein YhcN; stress response protein	biocide/metal	Unknown	BhsA/McbA family

			Hydrochloric acid (HCl) [class: Acid]					
yieF	BAC0541	P0AGE6	Chromium (Cr), Vanadium (V), Molybdenum (Mo), Methylene Blue [class: Thiazinium]	*Escherichia coli K12*	A chromate reductase. Reduces chromate (Cr(VI)) to Cr(III)	biocide/metal	Enzyme	SsuE family
zitB/ybgR	BAC0459	P75757	Zinc (Zn)	*Escherichia coli (strain K12)*	Zinc transporter ZitB; Involved in zinc efflux across the cytoplasmic membrane, thus reducing zinc accumulation in the cytoplasm and rendering bacteria more resistant to zinc; contributes to Zn++ haemostatis at low (non-toxic) concentrations; Appears to be selective for zinc, not conferring resistance to cobalt nor cadmium	metal	Efflux	Cation diffusion facilitator (CDF) transporter family
zntA/yhhO	BAC0461	P37617	Lead (Pb), Cadmium (Cd), Zinc (Zn)	*Escherichia coli (strain K12)*	Lead, cadmium, zinc and mercury-transporting ATPase ZntA; Involved in export of lead, cadmium, zinc and mercury; located in	metal	Enzyme	Cation transport ATPase (P-type) family

					inner cell membrane.			
zntR/yhdM	BAC046 2	P0ACS5	Zinc (Zn)	*Escherichia coli (strain K12)*	Zinc-responsive transcriptional regulator zntR; regulates the zntA	metal	Regulator	Contains 1 HTH merR-type DNA-binding domain
znuA/yebL	BAC046 3	P39172	Zinc (Zn)	*Escherichia coli (strain K12)*	High-affinity zinc uptake system protein ZnuA; Involved in the high-affinity zinc uptake transport system; located in periplasm	metal	Membrane Transporter	ABC superfamily, bacterial solute-binding protein 9 family
znuB/yebI	BAC046 4	P39832	Zinc (Zn)	*Escherichia coli (strain K12)*	High-affinity zinc uptake system membrane protein ZnuB; Involved in the high-affinity zinc uptake transport system; located in cell inner membrane	metal	Membrane Transporter	ABC-3 integral membrane protein family
znuC/yebM	BAC046 5	P0A9X1	Zinc (Zn)	*Escherichia coli (strain K12)*	Zinc import ATP-binding protein ZnuC; part of the ABC transporter complex ZnuABC involved in zinc import. Responsible for energy coupling to the transport system. Transcriptionally	metal	Binding protein	ABC transporter superfamily. Zinc importer family

					repressed by zur (zinc uptake regulator), in response to high extracellular zinc concentrations			
zraP	BAC0466	Q9L910	Zinc (Zn)	*Salmonella typhimurium (strain LT2/SGSC1412/ATCC 700720)*	Zinc resistance-associated protein ZraP; Undergoes a specific Zn++ induced cleavage to release a carboxy-terminal Zn++ binding region; located in periplasm; Binds zinc. Could be an important component of the zinc-balancing mechanism; located in periplasm	metal	Binding protein	ZraP family
zraR/hydH	BAC0467	P14375	Zinc (Zn)	*Escherichia coli (strain K12)*	Transcriptional regulatory protein ZraR; Member of the two-component regulatory system ZraS/ZraR. When activated by ZraS it acts in conjunction with sigma-54 to regulate the Expression of zraP. Positively autoregulates the Expression of the	metal	Regulator	Contains 1 response regulatory domain, 1 sigma-54 factor interaction domain

					zraSR operon.			
zupT/ygiE	BAC0469	P0A8H3	Zinc (Zn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Cadmium (Cd)	*Escherichia coli (strain K12)*	Zinc transporter ZupT; Mediates zinc uptake. May also transport other divalent cations such as copper and cadmium ions.	metal	Porter	ZIP transporter family, ZupT subfamily
zur/yjbK	BAC0470	P0AC51	Zinc (Zn)	*Escherichia coli (strain K12)*	Zinc uptake regulation protein zur; Acts as a negative controlling element, employing Zn2+ as a cofactor to bind the operator of the repressed genes (znuACB). Zinc uptake regulation protein.	metal	Regulator	Fur family

6 CONCLUSÃO

- Foi detectada a presença de *Salmonella* em todas as pisciculturas estudadas em Dourados, MS, podendo apresentar um emergente risco de Saúde Pública;
- *Salmonella* foi detectada em amostras de água de tanque, biofilme epilítico e peixe;
- Os peixes apresentaram-se como portadores assintomáticos que podem levar à disseminação no meio ambiente e podem representar risco para o homem quando consumidos peixes contaminados;
- A presença de *Salmonella* na água traz alerta sobre a possibilidade de atingirem outros corpos d'água ou solo após seu lançamento;
- Treze *S. enterica* foram isoladas e sorotipadas como *S.* Anatum, *S.* Minnesota, *S.* Panama e dois isolados classificados como *Salmonella enterica* subsp. *enterica*;
- As maiores taxas de resistência foram às sulfonamidas, trimetoprim, tetraciclina e estreptomicina;
- *Salmonella* MDR foi confirmada, o aumento no número de cepas de *Salmonella* MDR em pisciculturas parece ser um problema emergente para a disseminação da resistência entre as populações bacterianas;
- Todos os isolados de *Salmonella* apresentavam integrons de Classe 1, apresentando potencial fonte de disseminação de resistência ;
- O isolado sequenciado de *Salmonella* Panama possui resistência a aminoglicosídeos, bem como genes que codificam bombas de efluxo de múltiplas drogas e aqueles que conferem resistência a biocidas e metais pesados;
- O isolado sequenciado de *Salmonella* Panama possui diversos genes de virulência o que pode conferir maior compreensão dos mecanismos subjacentes à patogenicidade bacteriana associada a infecções em humanos e outros hospedeiros.
- Além de integrons de Classe I foi identificado o grupo de imcompatibilidade plasmidial IncF em *Salmonella* Panama e está entre os mais disseminados no mundo;
- Os plasmídeos IncFII é um dos mais frequentes plasmídeos de resistência a antimicrobianos, e sua presença está aparentemente ligada a pressão seletiva exercida pelo uso de antimicrobianos, pois sua prevalência é menor em bactérias não expostas a estes compostos;
- Os resultados deste trabalho demostram a disseminação de microrganismos resistentes a antibióticos na cadeia produtiva do pescado;

- Este trabalho sugere que biofilmes epilíticos podem ser utilizados como sentinela na detecção de *Salmonella* em tanques em fazendas peixe;
- Uma melhor compreensão dos mecanismos pelos quais a resistência antimicrobiana emerge e se espalha na aqüicultura deve nos permitir projetar estratégias para reduzir a progressão e implementar medidas de controle para proteger a saúde humana e animal e o meio ambiente.

7 ANEXOS

-Artigos Publicados

Article

A Promising Copper(II) Complex as Antifungal and Antibiofilm Drug against Yeast Infection

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Abstract: The high mortality rate of candidemia and the limited option for the treatment of *Candida* spp. infection have been driving the search for new molecules with antifungal property. In this context, coordination complexes of metal ions and ligands appear to be important. Therefore, this study aimed to synthesize two new copper(II) complexes with 2-thiouracil and 6-methyl-2-thiouracil ligands and to evaluate their mutagenic potential and antifungal activity against *Candida*. The complexes were synthesized and characterized by infrared vibrational spectroscopy, CHN elemental analysis, UV-Vis experiments and ESI-HRMS spectrometry studies. The antifungal activity was evaluated by broth microdilution against 21 clinical isolates of *Candida* species. The mutagenic potential was evaluated by the Ames test. The complexes were $Cu(Bipy)Cl_2$(thiouracil) (Complex **1**) and $Cu(Bipy)Cl_2$(6-methylthiouracil) (Complex **2**). Complex **1** showed fungicidal and fungistatic activities against all isolates. Furthermore, the Minimum Inhibitory Concentration (MIC) from 31 to 125 μg/mL and inhibition percentage of 9.9% against the biofilms of *C. krusei* and *C. glabrata* were demonstrated. At the concentrations tested, complex **1** exhibited no mutagenic potential. Complex **2** and the free ligands exhibited no antifungal activity at the concentrations evaluated. Since complex **1** presented antifungal activity against all the tested isolates and no mutagenic potential, it could be proposed as a potential new drug for anti-*Candida* therapy.

Keywords: antifungal; ames test; biofilm; copper(II); 2-thiouracil

1. Introduction

Candida species are one of the most important opportunistic fungal pathogens worldwide. These yeasts are part of the normal microbiota in the human gastrointestinal, respiratory, and

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ORIGINAL RESEARCH

Synergy of Biodegradable Polymer Coatings with Quaternary Ammonium Salts Mediating Barrier Function Against Bacterial Contamination and Dehydration of Eggs

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Abstract
Eggs are an important, low-cost, high-protein food with a balanced nutritional composition. The sanitary quality of commercial eggs is a bottleneck that needs to be overcome through the use of technologies that guarantee the safety of the product. This work aimed to prepare, characterize, and evaluate the coating of commercial eggs by chitosan filmogenic solutions and 1st- and 5th-generation quaternary ammonium salt components (QACs). Egg shell properties such as thickness, morphology, and wettability were analyzed by scanning electron microscopy (SEM),TG/DSC, pH_{PZC}, contact angle, color, and weight loss. Determinations of the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) with *Salmonella* spp., *Escherichia coli*, and *Staphylococcus aureus* were performed, as well as proliferation tests on eggshell surfaces. The results show a synergistic effect of the biopolymer in terms of antibacterial activity against all microorganisms tested. The composite of chitosan and ammonium quaternary ammonium films offers physical and biological safety to guarantee the integrity and microbiological quality of commercial eggs. Egg coating using bioactive chitosan films was shown to be a suitable method of preservation and a physical and microbiological barrier to minimize egg quality loss during storage, as well as to increase shelf life.

Keywords Synergistic effect, biomimetic and intelligent coatings · Self-repairing films in eggs · Polymer protecting eggs · Increased shelf life · Chitosan filmogenic solution · Intelligent egg coating

Taiane Almeida e Silva, Luiz Fernando Gorup and Renata Pires de Araújo contributed equally to this work.

Synopsis
Towards a green biopackaging material for eggs, natural polymeric antimicrobial coatings were developed and investigated to improve sanitary quality and extend shelf life.

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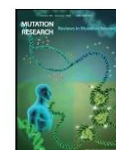

Review

Mutagenic potential of medicinal plants evaluated by the Ames *Salmonella*/microsome assay: A systematic review

Fabiana Gomes da Silva Dantas[a,b], Pamella Fukuda de Castilho[a], Adriana Araújo de Almeida-Apolonio[a], Renata Pires de Araújo[b,c], Kelly Mari Pires de Oliveira[a,b,*]

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ABSTRACT

The Ames test has become one of the most commonly used tests to assess the mutagenic potential of medicinal plants since they have several biological activities and thus have been used in traditional medicine and in the pharmaceutical industry as a source of raw materials. Accordingly, this review aims to report previous use of the Ames test to evaluate the mutagenic potential of medicinal plants. A database was constructed by curating literature identified by a search on the electronic databases Medline (*via* Pubmed), Science Direct, Scopus, and Web of Science from 1975 to April 2020, using the following terms: “genotoxicity tests” OR “mutagenicity tests” OR “Ames test” AND “medicinal plants.” From the research, 239 articles were selected, including studies of 478 species distributed across 111 botanical families, with Fabaceae, Asteraceae and Lamiaceae being the most frequent. It was identified that 388 species were non-mutagenic. Of these, 21% (83/388) showed antimutagenic potential, most notable in the Lamiaceae family. The results also indicate that 18% (90/478) of the species were mutagenic, of which 54% were mutagenic in the presence and absence of S9. Strains TA98 and TA100 showed a sensitivity of 93% in detecting plant extracts with mutagenic potential. However, the reliability of many reviewed studies regarding the botanical extracts may be questioned due to technical issues, such as testing being performed only in the presence or absence of S9, use of maximum doses below 5 mg/plate and lack of information on the cytotoxicity of tested doses. These methodological aspects additionally demonstrated that a discussion about the doses used in research on mixtures, such as the ones assessed with botanical extracts and the most sensitive strains employed to detect the mutagenic potential, should be included in a possible update of the guidelines designed by the regulatory agencies.

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SHORT COMMUNICATION

Cytotoxicity, mutagenicity and acute oral toxicity of aqueous *Ocotea minarum* leaf extracts

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ABSTRACT

Ocotea minarum (Nees & Mart.) Mez., a native species of Brazil, is used in the treatment of infections and oxidative stress; however, there is no scientific evidence of its toxicological characteristics. We assessed the cytotoxicity, mutagenic activity, and acute oral toxicity of the aqueous extract isolated from *O. minarum* leaves. The cytotoxicity of this extract was evaluated in tumour and non-tumour cell lines, while the Ames test with a *Salmonella* Typhimurium was used to determine the mutagenic activity. Wistar rats received a single 2 g/kg dose as part of an LD_{50} toxicity assessment. Our results showed that the aqueous extract of *O. minarum* leaves did not present cytotoxic and mutagenic properties and was not toxic, with an LD_{50} greater than 2 g/kg. Therefore, the *O. minarum* extracts are pharmacologically safe and can continue to be investigated for the development of new drugs and herbal medicines.

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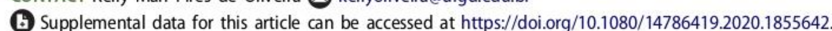

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Prezados Inventores,

Parabéns! Seu pedido de patente foi depositado com sucesso.

Protocolado junto ao Instituto Nacional da Propriedade Industrial (INPI).
A partir de agora é permitido fazer divulgações e publicações.
Solicitamos que nos informem sobre possíveis interessados para transferência da tecnologia.

PROTOCOLO DE DEPÓSITO DE PEDIDO DE PATENTE:
Número do Processo: BR 10 2019 014216 2
Título: COBERTURAS E FILMES BIOATIVOS PARA CONTROLE SANITÁRIO E PRESERVAÇÃO DE OVOS
Categoria: Patente de Invenção
Data de depósito: 09-07-2019

Em caso de adequações técnicas durante o período de análise pelo INPI entraremos em contato.

Atenciosamente,

Honorivaldo Albuquerque

PROTOCOLO DE DEPÓSITO DE PEDIDO DE PATENTE

Uma confirmação de leitura foi enviada para este remetente.

NIPI (Núcleo de Inovação UFGD) <nipi@ufgd.edu.br>
Seg, 22/07/2019 09:42
Para: Você; Kelly Mari Pires de Oliveira; aaraujo.a@hotmail.com; marirchang@yahoo.com.br; claudia@uems.br; fabianasilva@ufgd.edu.br

Prezadas Inventoras,

Parabéns! Seu pedido de patente foi depositado com sucesso.

Protocolado junto ao Instituto Nacional da Propriedade Industrial (INPI).
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PROTOCOLO DE DEPÓSITO DE PEDIDO DE PATENTE:
Número do Processo: BR 10 2019 015012 2
Título: COMPOSIÇÃO PARA TRATAMENTO DE CANDIDÍASES
Categoria: Patente de Invenção
Data de depósito: 22/07/2019

Em caso de adequações técnicas durante o período de análise pelo INPI entraremos em contato.