UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO" CÂMPUS BOTUCATU
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

INFLUÊNCIA DE UM SISTEMA DE PRODUÇÃO DE PESCADO NA RESISTÊNCIA DE *Escherichia coli* AOS ANTIMICROBIANOS

STHÉFANY DA CUNHA DIAS

Botucatu – São Paulo
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Orientador: Prof. Dr. Juliano Gonçalves Pereira

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LISTA DE ABREVIAÇÕES

AIEC - *Escherichia coli* aderente invasiva

AMC - Amoxicilina com ácido clavulânico

AMO - Amoxicilina

APT - Água peptonada tamponada a 1%

ATM - Aztreonam

AZI - Azitromicina

BHI - Infusão de cérebro e coração

CAZ – Ceftazidima

CIP – Ciprofloxacina

CLO - Cloranfenicol

CPM - Cefepime

CTF - Ceftiofur

CTX - Cefotaxima

DAEC - *Escherichia coli* difusamente aderente

DNA - Ácido desoxirribonucleico

EAEC - *Escherichia coli* enteroagregativa

EIEC - *Escherichia coli* enteroinvasiva

EPEC - *Escherichia coli* enteropatogênica

EPM - Escola paulista de medicina

ETEC - Escherichia coli enterotoxigênica

FAMEV-UFU - Faculdade de Medicina Veterinária e Zootecnia da Universidade Federal de Uberlândia

GEN - Gentamicina

IPM - Imipenem

MDR - Multirresistente

MILi - Motilidade indol lisina

SISBI-POA - Sistema brasileiro de inspeção de produtos de origem animal

STEC - *Escherichia coli* produtora de toxina shiga

SUT - Sulfametoxazol com trimetoprim

t - toneladas

TET - Tetraciclina

LISTA DE TABELAS

LISTA DE FIGURAS

SUMÁRIO

DIAS, S.C. **Influência de um sistema de produção de pescado na resistência de *Escherichia coli* aos antimicrobianos.** Botucatu, 2021. 66p. Dissertação (Mestrado) - Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

RESUMO

A intensificação da produção tem sido uma realidade na aquicultura, o que aumenta a densidade de criação, estresse e deixa os animais mais susceptíveis a doenças, aumentando o risco de comprometimento da qualidade microbiológica do produto final. Um dos riscos é a presença de patógenos zoonóticos como *Escherichia coli*, micro-organismo associado a surtos alimentares. Além da patogenicidade dos micro-organismos, a resistência das bactérias aos antimicrobianos é uma preocupação mundial, que compromete o tratamento de seres humanos e reduz as possibilidades de terapêutica ideal. Desse modo, o trabalho teve como objetivo caracterizar o perfil de resistência de isolados de *E. coli* obtidos de uma cadeia produtiva de tilápias com amostras representativas da produção animal, ambiente industrial e produto final. *E. coli* foi identificada em todos os pontos coletados, sendo que das 240 amostras analisadas foram encontrados 61 perfis de resistência, sendo 21 deles de multirresistência. Houve uma redução de 31% da presença do patógeno ao longo da cadeia de produção ($P< 0,0001$) quando comparados carcaça pós-sangria e produto final. Os pontos de coleta referentes à produção animal apresentaram maior número de cepas multirresistentes, com isolados resistentes a até 8 bases de antimicrobianos. A pesquisa é um alerta sobre o uso indiscriminado de antimicrobianos, a multirresistência presente na produção animal e a importância de inquéritos epidemiológicos, monitoramento e investigação quanto ao risco para saúde pública, animal e meio ambiente. Também é reforçada a necessidade de cautela no uso de antimicrobianos em ambientes de produção e estímulos para utilização de tecnologias como meios alternativos.

Palavras-chave: antibióticos, sensibilidade microbiana, tilápia.

DIAS, S.C. **Influence of the fish production system on the distribution of antibiotic resistance in *Escherichia coli.*** Botucatu, 2021. 66p. Dissertation (MSc Degree) - Faculdade de Medicina Veterinária e Zootecnia, Campus de Botucatu, Universidade Estadual Paulista.

ABSTRACT

The intensification of production has been a reality in aquaculture, which increases the density of creation, stress and leaves the animals more susceptible to diseases, increasing the risk of compromising the microbiological quality of the final product. One of the risks is the presence of zoonotic pathogens such as *Escherichia coli,* a microorganism associated with food outbreaks. In addition to the pathogenicity of microorganisms, the resistance of bacteria to antimicrobials is a worldwide concern, which compromises the treatment of human beings and reduces the possibilities of optimal therapy. Thus, the objective of this work was to characterize the resistance profile of *E. coli* isolates obtained from a tilapia production chain with representative samples of animal production, industrial environment and final product. *E. coli* was identified at all points collected, and from the 240 samples analyzed, 61 resistance profiles were found, 21 of which were multi-resistance. There was a 31% reduction in the presence of the pathogen along the production chain ($P< 0.0001$) when comparing post-bleed carcass and final product. The collection points referring to animal production showed a greater number of multiresistant strains, with isolates resistant to up to 8 antimicrobial bases. This is a warning about the indiscriminate use of antimicrobials, the multidrug resistance present in animal production and the importance of epidemiological surveys, monitoring and investigation regarding the risk to public, animal and environment health. The study reinforces the need for caution in the use of antimicrobials in production environments and encourages the use of technologies as alternative means.

Keywords: antibiotics, microbial sensitivity, tilapia.

CAPÍTULO 1

1. INTRODUÇÃO

O Brasil é reconhecido mundialmente por seu potencial agroexportador, movimentando a economia do país e gerando emprego e renda nas diversas cadeias de trabalho envolvidas no agronegócio. Contribuindo para a segurança alimentar em diversas regiões do mundo, o pescado representa uma importante fonte de proteína animal (FAO, 2018). A produção dessa matriz alimentar é um mercado em crescimento, sendo que a piscicultura atingiu a 800.000 toneladas produzidas em 2020 no Brasil (PEIXE BR, 2021), evidenciando a importância e crescimento dessa cadeia e seus impactos econômicos e sociais, uma vez que cerca de 8% da população mundial é dependente desse setor (FAO, 2018).

No cenário da piscicultura nacional, destaca-se a produção de tilápia (*Oreochromis* spp.). A espécie representa cerca de 60 % da produção de cultivo brasileira, mantendo o país como o quarto maior produtor mundial (PEIXE BR, 2020). A intensificação do sistema é um reflexo direto da globalização, que tem uma demanda crescente por produtos de origem animal (DAWOOD et al., 2016). Esse aumento está cada vez mais associado à produção de cultivo de modo intensivo, com o intuito de fortalecer o potencial produtivo e econômico dessa matriz alimentar (REBELATO et al., 2015). Com isso, a adoção de medidas de biosseguridade e boas práticas agropecuárias são essenciais para a prevenção de surtos de doenças que envolvam a cadeia e que possam comprometer a saúde do consumidor devido ao caráter zoonótico (VAN BOECKEL et al., 2017).

Dentre os patógenos com potencial zoonótico destaca-se *E. coli*. Essa bactéria já foi encontrada em aquiculturas, é associada à resistência à maioria dos antibióticos utilizados na clínica médica e se caracteriza pela eficiência em disseminar genes de resistência (LIHAN et al., 2021). Desse modo, o trabalho tem como objetivo avaliar a resistência de *E. coli* oriundas dos animais, ambiente industrial e produto final de uma cadeia produtiva de tilápias quando submetidas a testes fenotípicos com diferentes classes de antibióticos de relevância para a saúde humana, contemplando uma abordagem em Saúde Única.

2. REVISÃO DE LITERATURA

2.1. PRODUÇÃO DE PESCADO

O termo “pescado” segundo o Regulamento de Inspeção Industrial e Sanitária de Produtos de Origem Animal (RIISPOA), abrange os peixes, crustáceos, anfíbios, moluscos, répteis, equinodermos e demais animais aquáticos utilizados na alimentação humana (BRASIL,2017). Ou seja, “pescado” é usado de forma genérica para qualquer animal que seja produzido total ou parcialmente em ambiente aquático. Dentre os integrantes dessa classificação, os peixes se destacam com um importante papel na economia mundial como fonte de alimentação, comércio e subsistência (FAO, 2020).

O consumo de pescado cresce gradualmente e tem-se a perspectiva de que em 2030 atinja o consumo de 20kg por pessoa ao ano. No aspecto econômico, a piscicultura gera renda para 520 milhões de pessoas, cerca de 8% da população mundial, evidenciando a importância do setor no cenário global (FAO, 2020). Além disso, o peixe é uma proteína de origem animal que ganha destaque por sua composição com baixa porcentagem de gordura e rica em nutrientes essenciais como vitaminas, aminoácidos e minerais, contribuindo para a segurança alimentar em diversos locais (FAO, 2020). Nesse contexto, a tilápia (*Oreochromis* spp.) é classificada como a terceira espécie de peixe mais produzido em todo o mundo (FAO, 2020).

A piscicultura brasileira atingiu a produção de 800.000 toneladas em 2020, segundo melhor desempenho desde 2014 (PEIXE BR, 2021). A tilápia se destaca como o principal peixe de cultivo e representa cerca de 60% da produção nacional, atingindo 486.000 toneladas (PEIXE BR, 2021). Geograficamente, a produção é concentrada na região sul do país e o estado do Paraná é o maior produtor nacional (166.000 t), seguido de São Paulo (70.500 t), Minas Gerais (42.100 t) e Santa Catarina (40.059 t) (PEIXE BR, 2021). Desse modo, o Brasil mantém a tradição de grande produtor de proteína animal e, com isso, se consolida como o quarto maior produtor de tilápias do mundo, atrás apenas da China, Indonésia e Egito (PEIXE BR, 2021).

Em relação ao mercado internacional, a exportação apresenta números crescentes e teve um aumento de 8% em relação ao ano anterior, passando de 6.201 t para 6.680 t (PEIXE BR, 2021). Dentre os produtos brasileiros exportados, os filés frescos e refrigerados representam 45% do volume das exportações, seguido dos óleos e gorduras (18%) e peixes

congelados (15%) (PEIXE BR, 2021). A tilápia é a principal espécie dos produtos de exportação e representa 88% da demanda internacional, cujos principais destinos são Estados Unidos, Chile e China (PEIXE BR, 2021).

A aquicultura segue avançando em tecnologia, pesquisa, produção e investimentos em sustentabilidade. No entanto, para o fortalecimento do setor é de suma importância o monitoramento e controle de problemas de caráter sanitário. Desse modo, as boas práticas e biosseguridade devem estar presentes em todas etapas da produção até o produto final (PEIXE BR, 2021). Dentre os desafios a serem enfrentados na aquicultura, destacam-se a melhoria sanitária das produções e alternativas em substituição ao uso de antimicrobianos (FAO, 2020).

2.2. ANTIMICROBIANOS E AQUICULTURA

A aquicultura se caracteriza por uma produção diversificada, na qual a água é um elemento essencial para a concretização da cadeia produtiva (MELLO et al., 2006). E, a intensificação do sistema é um reflexo direto da globalização, que tem uma demanda crescente por produtos de origem animal (DAWOOD et al., 2016). Com isso, a adoção de medidas de biosseguridade e boas práticas agropecuárias são essenciais para a prevenção de surtos de doenças que comprometam a cadeia produtiva e que possam alcançar o consumidor final (VAN BOECKEL et al., 2017).

O rápido aumento da produção causa preocupação quanto à saúde e qualidade dos peixes produzidos, sendo que o uso de antimicrobianos tem o intuito de controlar surtos e manter a sanidade da produção (SANTOS et al., 2018). Nesse contexto, os antimicrobianos são utilizados amplamente na agroindústria sob diversas modalidades. Dentre elas tem-se a forma profilática, medida de caráter preventivo e a terapêutica, tratamento individualizado para controlar uma infecção ativa. Também existe a forma de uso metafilática, que tem o intuito de conter um surto ou minimizar os sinais clínicos de uma doença em uma população com a utilização de subdose em relação ao tratamento convencional; e a utilização como promotor de crescimento (SCHWARZ et al., 2001).

As projeções para 2030 evidenciam a aquicultura como a consumidora de 5,7% do uso global de antibióticos, com aumento de 33% entre 2017 e 2030. E, diante desse cenário, com produções animais altamente intensificadas e uso excessivo de antimicrobianos, tem-se o

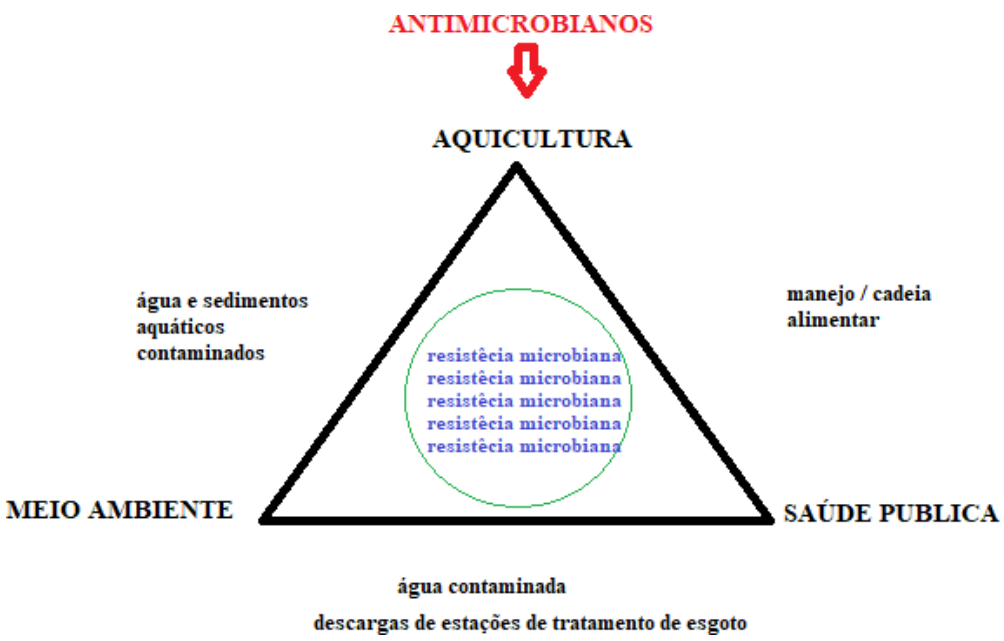
risco da seleção de micro-organismos resistentes, temática que se mostra como um desafio global (SCHAR et al., 2020). Este é um problema relevante, pois grande parte das bases utilizadas na medicina veterinária são comuns ao uso na medicina humana, aumentando a probabilidade de ocorrência de resistência em micro-organismos associados a humanos e animais e, consequentemente, aumentando o risco à saúde dos envolvidos (EMA, 2014).

Essa situação evidencia uma crise sanitária mundial que estima-se causar 700.000 mortes anualmente e com projeções de 10.000.000 de mortos em 2050 (WATTS et al., 2017). Em vista disso, a resistência microbiana gera prejuízos diretos na produção com a alteração do microbioma local ou indiretamente com patógenos da cadeia produtiva atingindo os seres humanos e dificultando a seleção de uma terapêutica eficaz (CABELLO et al., 2016).

Os antimicrobianos podem ser de amplo espectro, ou seja, sua ação pode abranger uma gama diversa de patógenos ou de ação estrita, na qual a ação é sob um agente ou classe específica (SCHWARZ et al., 2001). Dentro da aquicultura, tem-se a intensificação da produção assim como outras cadeias animais. No entanto, o uso de antibióticos no sistema aquático expõe o ambiente numa escala muito maior devido o veículo de distribuição dos medicamentos ser a água (SCHAR et al., 2020). Nesse cenário, cerca de 80% das drogas utilizadas na aquicultura são encontradas no meio produtivo com sua bioatividade intacta, corroborando para alteração do microbioma aquático (CABELLO et al., 2013).

Há mais de 30 anos, sabe-se da interação entre microbioma intestinal dos animais de produção e de seus tratadores, com influência direta sobre a resistência destas a partir do uso de antimicrobianos como promotores de crescimento (LEVY et al., 1976). Na indústria de alimentos já foram encontrados indícios da contaminação de alimentos por seus manipuladores, com implicação para a saúde pública (ELTAI et al., 2018). Então, de um modo geral, o uso de fármacos na agroindústria e seus resíduos ambientais impactam no ecossistema aumentando a pressão de seleção de micro-organismos e a possibilidade de alcance de patógenos resistentes até o homem (Figura 1) (SCHAR et al., 2020).

Figura 1 - Uso de antimicrobianos na aquicultura e seus impactos na saúde única.



Fonte: SANTOS et al., 2018 (adaptado)

Nesse contexto, com a aquicultura utilizando diversas toneladas de antimicrobianos para uso terapêutico e profilático, tem-se o aumento da pressão seletiva, o que corrobora para a mutação bacteriana, seleção de genes de resistência e troca de material genético através da alteração do microbioma local e resposta adaptativa ao meio (TOPP et al., 2018). A interação de agente, meio ambiente e hospedeiro, constitui a tríade epidemiológica e consiste na interação básica que ocorre e integra fatores que influenciam doenças de caráter zoonótico (CABELLO et al.,2016). Os gêneros bacterianos *Enterococcus*, *Escherichia*, *Campylobacter*, *Salmonella* e *Clostridium* já foram evidenciados como comensais da flora intestinal animal, mas com potencial de causar doenças graves em humanos sendo a via alimentar a principal origem de infecção (MARSHALL et al., 2011).

Enfermidades humanas ocasionadas por agentes veiculados por alimentos têm chamado a atenção devido ao aumento da resistência dos patógenos, ocasionando dificuldade para obter resultados satisfatórios com as terapêuticas convencionais (MILLER et al., 2018). Os produtos de origem animal associados a esse risco são diversos, dentre os quais se destacam o leite, carne suína, bovina e de aves, além do pescado (MARSHALL et al., 2011). Com isso, tem-se a evidência de que a interação entre os diferentes elos da cadeia produtiva e alimentar

podem provocar prejuízos a todos os envolvidos, requerendo a adoção de boas práticas e biosseguridade cada vez mais rígidas.

A ciência já possui evidências concretas do papel seletor que os antibióticos exercem na propagação de agentes resistentes, sejam eles patogênicos ou comensais (CABELLO et al., 2013). Existem políticas globais que elencam tópicos que são considerados essenciais para conter o consumo de antimicrobianos na produção animal: limites para o uso, redução ao consumo de produtos de origem animal e taxas sobre os fármacos veterinários utilizados (VAN BOECKEL et al., 2017). Essas medidas surgiram em reação ao principal consumidor de antimicrobianos e gerador de resistência: a produção animal, responsável por cerca de 80% do consumo de antibióticos em todo o mundo (VAN BOECKEL et al., 2017).

A evidência dessa situação é a presença de fármacos, bactérias resistentes e seus genes de resistência presentes em solo de área de produção animal. Isso sugere uma persistência ambiental encontrada em todo o sistema produtivo local, com o risco de exposição do microbioma humano em diversas vias. Quando o foco de análise é a aquicultura, a literatura destaca que existe similaridade no padrão molecular de resistência encontrado neste tipo de produção e bactérias causadoras de doenças no homem como *E. coli*, corroborando para o caráter abrangente da temática (TOMOVA et al., 2015).

2.3. MECANISMOS DE AÇÃO DOS ANTIMICROBIANOS

É evidente a correlação direta entre a cadeia produtiva de produtos de origem animal e o uso de antimicrobianos nas suas diversas funções. No entanto, a escolha do antibiótico depende de variáveis a serem analisadas para uma escolha assertiva, dentre elas estão: classe do antibiótico, tempo de meia vida, espectro de ação, concentração, tecido-alvo e diagnóstico (EYLER et al., 2019). Na piscicultura, utilizam-se altas concentrações de antibióticos, com uma diversidade de bases químicas (HAN et al., 2021). Dentre elas destacam-se os aminoglicosídeos, quinolonas, beta-lactâmicos, sulfonamidas, macrolídeos, anfenicóis, dentre outros (CHEN et al., 2020).

Em relação às diversas classes de antibióticos, os beta-lactâmicos são uma família de drogas que possuem conformação química estrutural em comum, na qual um anel b-lactâmico se faz presente (LIMA et al., 2020). Eles atuam inibindo a última etapa da síntese do peptidoglicano da parede celular bacteriana, sendo que essa é uma forma estrutural

comum entre as penicilinas, as cefalosporinas e carbapenêmicos (MOHR, 2016). As cefalosporinas de primeira geração são uma boa escolha no tratamento de bacteremias ocasionadas por gram-positivos, com resultados melhores quando associados a outrosbeta-lactâmicos (EYLER et al., 2019). Já as cefalosporinas de terceira e quarta geração, comoa ceftazidima, ceftiofur e cefotaxima são a opção de escolha para o tratamento contra gram-negativos (LIMA et al., 2020). Dentre os patógenos sensíveis às cefalosporinas de terceira geração destacam-se *E. coli*, *Klebsiella*, *Citrobacter*, *Proteus* e *Morganella* (BARRIERE et al., 1984). Alterações estruturais no anel b-lactâmico originaram os carbapenêmicos. Essa classe tem o imipenem como o principal representante e possuem amplo espectro, são bem tolerados e abrangem os micro-organismos produtores de b-lactamase (EYLER et al., 2019).

Glicopeptídeos, como a vancomicina, possuem atividade contra gram-positivas e tem ação bacteriostática, inibindo a síntese de precursores da parede celular bacteriana (EYLER et al., 2019). Já os lipopeptídeos, classe que tem a daptomicina como membro exclusivo, tem atividade bactericida e age alterando a despolarização de membranas bacterianas de agentes gram-positivos (EYLER et al., 2019). Os lipoglicopeptídeos surgiram em 2009, com estrutura semelhante à dos glicopeptídeos. Possuem atividade bactericida e devem ser usados com cautela devido ao potencial nefrotóxico (EYLER et al., 2019).

Os aminoglicosídeos são usados desde 1940 para tratamento de infecções e se caracterizam por seu amplo espectro (RAMIREZ et al., 2017). São representantes da classe a gentamicina e amicacina, se caracterizam pelo potencial bactericida e atuam inibindo a síntese proteica de bactérias, ligando-se a unidade ribossomal 30S (MOHR, 2016). Deixaram de ter importância significativa com o surgimento de novas bases químicas que ocasionam menos efeitos colaterais (BECKER et al., 2013). São potencialmente tóxicos, sendo utilizados em doses baixas, porém eficazes contra patógenos resistentes (EYLER et al., 2019).

Fluoroquinolonas, como a ciprofloxacina, são de amplo espectro, boa penetração tecidual e atuam na inibição de síntese de DNA, inativando a DNA girase em gram negativas e DNA topoisomerase em gram-positivas (EYLER et al., 2019). Da classe dos anfenicóis, o cloranfenicol se destaca por seu amplo espectro, com atividade sob a síntese proteica bacteriana, inibindo a formação de ligações peptídicas (MOHR, 2016). Sulfonamidas atuam por competição de sítio de ligação de enzimas bacterianas. É utilizado em associação com

trimetoprim, inibidor de enzimas bacterianas (EYLER et al., 2019).

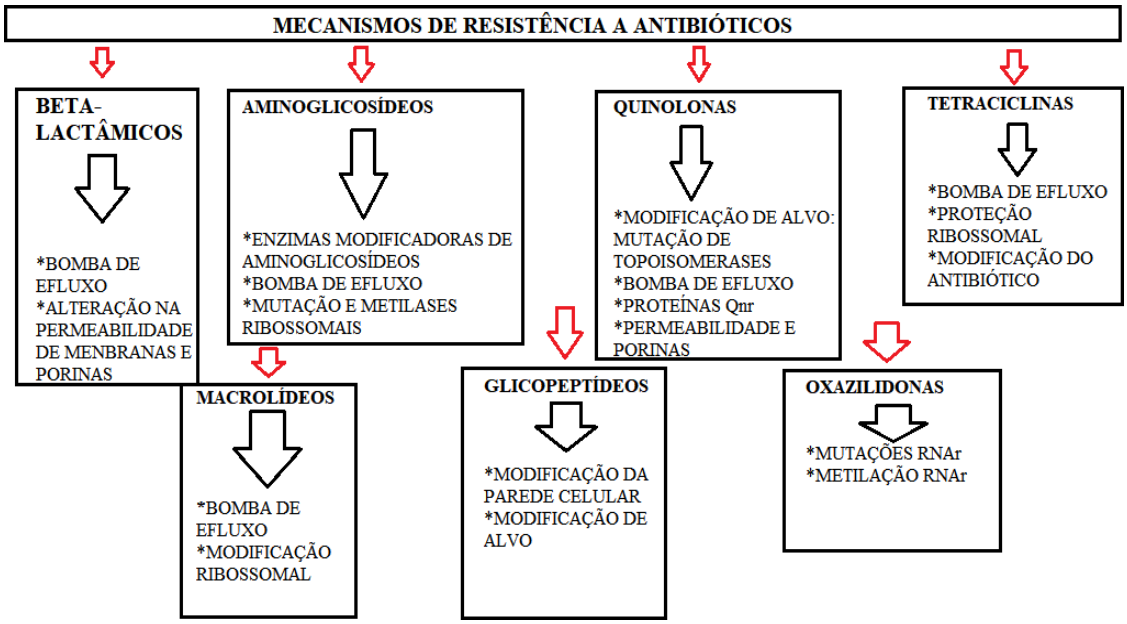
As tetraciclinas como a oxitetraciclina e a tetraciclina tem atuação bacteriostática, por inibição de síntese proteínas, com atuação no sítio ribossomal 30S. Podem ser produzidas naturalmente como a clortetraciclina ou sintetizadas como a doxiciclina (NGUYEN et al., 2014). Possuem amplo espectro, atuando contra bactérias gram-positivas e negativas, micoplasmas, riquétsias e protozoários (MOHR, 2016). Por ocasionar poucos efeitos adversos, foi amplamente utilizado na medicina humana e veterinária, porém o surgimento de resistência limitou o uso (CHOPRA et al., 2001).

Macrolídeos, como a azitromicina, são antibióticos usados há mais de 60 anos e são exemplo de sucesso quanto ao mecanismo de ação utilizado (VÁZQUEZ LASLOP et al., 2018). São estruturas compostas por ligação de anel de lactona macrocíclica a um açúcar aminoglicosídico (BAKHEIT et al., 2014). Possuem amplo espectro, sendo que o mecanismo de ação consiste em efeito bacteriostático por ligação à subunidade 50S do ribossomo e inibição da síntese bacteriana (MOHR, 2016). O uso dessa classe é preferível em infecções por bactérias gram-positivas, uma vez que a penetração nas porinas e, consequentemente, a resposta frente ao agente é abaixo do esperado quando o desafio são micro-organismos gram-negativos (BRISSON NOEL et al., 1988). Já as lincosamidas, como a clindamicina, são fórmulas bacteriostáticas, que se ligam ao RNA 23S dos ribossomos bacterianos e, consequentemente, impede a síntese proteica (MOHR, 2016).

2.4. MECANISMOS DE RESISTÊNCIA AOS ANTIMICROBIANOS

A resistência microbiana é uma das principais causas de terapêuticas não responsivas (ISLAM et al., 2019). Ela se refere à habilidade de um determinado micro-organismo se manter viável e crescer na presença de uma droga (WACLAW, 2016). O que se observa são diversos mecanismos usados pela maquinaria microbiana com o intuito de evadir do efeito dos antibióticos (PARTRIGDE et al., 2018). Para a caracterização de um perfil de resistência, podem ser feitas diversas mensurações, sendo que baixos níveis de sensibilidade microbiana tendem a serem relacionados a mediação por genes (seja por mutação ou combinação de um ou vários genes) que codificam enzimas que degradam ou inativam fármacos por diferentes vias (figura 2) (WACLAW, 2016).

Figura 2 - Classes de antimicrobianos e respectivos mecanismos de resistência.



Fonte: PONTES et al., 2018 (Adaptado)

Diante disso, os mecanismos de troca genética mais comuns entre bactérias são a conjugação, transdução e transformação (GOSH et a., 2020). A interação de todos esses fatores contribui para a modificação do microbioma e contribuem para uma disseminação mais rápida da resistência microbiana (PARTRIGDE ET a., 2018). Por vezes, uma única modificação no DNA bacteriano é capaz de originar resistência a determinado antimicrobiano (WAGLECHNER et al., 2017).

A conjugação consiste na troca de material genético pelo contato direto entre células (KHAN et al., 2018). Exemplo de mecanismo de conjugação é troca de DNA por plasmídeos, elementos genéticos móveis extra-cromossomais que são importantes veículos para a disseminação de genes de resistência em bactérias, com destaque para a resistência a beta-lactâmicos (ARIAS et al., 2003). A maioria dos genes de resistência é distribuída por elementos genéticos móveis como os plasmídeos, promovendo a mobilidade do material genético de modo intra e intercelular (PARTRIGDE et a., 2018).

Já a transdução é a modalidade de troca de material genético mediada por um fago. São classificadas em generalizada, no qual qualquer sequência genética pode ser transferida ou especializada, com a transferência de um segmento genético específico (TOUCHON et al., 2017). De modo geral, os transposons e sequências de inserção atuam movimentando material genético para diferentes locais dentro de uma mesma célula bacteriana

(PARTRIGDE et a., 2018).

A transformação é a forma na qual a aquisição de genes se dá pela absorção de DNA disperso no meio ambiente (KHAN et al., 2018). Esse é um modo de transferência horizontal de genes, assim como conjugação e transdução (NING et al., 2018). O que se observa é que taxas de conjugação e transdução são maiores in vivo que in vitro. No entanto, a mensuração dessas taxas para a transformação ainda não tem definições claras (DURÃO et al., 2018).

Ademais aos mecanismos citados, a persistência bacteriana também pode ser considerada um mecanismo de indução de resistência e consiste numa resposta de uma determinada população frente a um agente estressor, cujo crescimento é reduzido ou interrompido (GOSH et al., 2020). As células bacterianas persistentes não têm modificações genéticas, porém respondem facilmente a alterações ambientais e, com isso, podem se manter estáveis quando submetidas à terapia antibiótica (PONTES et al., 2018).

Além dos mecanismos tradicionais de resistência, a estrutura morfológica de bactérias gram-negativas é mais preocupante quando comparada às bactérias gram-positivas. Devido a sua porção lipofílica, os antibióticos com estrutura hidrofílica não conseguem penetrar no micro-organismo, sendo que qualquer alteração conformacional pode ocasionar o surgimento de resistência (BREIJYEH et al., 2020).

Frequentemente um micro-organismo apresenta mais de um gene de resistência a antimicrobianos, podendo expressá-los ou não (MCGOWAN, 2006). Cepas caracterizadas como extensivamente resistentes são resistentes a pelo menos uma categoria de droga , jáas multirresistentes a pelo menos um antibiótico em três ou mais classes . Por fim, define-se por panrresistente o isolado que é resistente a todas as bases testadas (JEONG et al., 2007).

Entre as gram-negativas, a resistência é preocupante devido à habilidade de produção de beta-lactamase de espectro estendido (ESBL), enzimas que conferem maior resistência às cepas devido a capacidade de hidrólise de beta-lactâmicos (FARIÑAS et al., 2013). ESBL vêm se disseminando rapidamente e estão associadas a causas multifatoriais de disseminação como a produção animal, ambiente hospitalar, internacionalização de produtos alimentícios e transmissão direta dentro do ambiente familiar (DOI et al., 2017). *E. coli* é um micro-organismo dentre os gram-negativos com importante participação na disseminação de genes de resistência (MARTINEZ-MARTINEZ et al., 2010).

2.5. *Escherichia coli* – IMPORTÂNCIA E CARACTERÍSTICAS

Patógenos zoonóticos como *E. coli, Salmonella* spp. E *Staphylococcus* spp. Podem estar inseridos nos sistemas de produção. E, por sua vez, podem comprometer a inocuidade dos produtos de origem animal, impactando diretamente no consumidor final (DHAMA et al., 2013). *E. coli* já foi encontrada em aquiculturas associada a resistência à maioria dos antibióticos utilizados na clínica médica e se caracteriza pela eficiência em disseminar genes de resistência (PREENA et al., 2021).

Na Ásia já existem evidências de *E. coli* produtora de beta-lactamase de espectro estendido (ESBL) na microbiota interstinal de cerca de 50% da população humana, em águas residuárias de produção animal e em peixes para consumo humano (HARADA, 2018; HASSAN et al., 2021). Nesse aspecto, é evidente o risco da água contaminada para a biosseguridade do setor agropecuário, produto final e saúde humana, se comportando como um importante como veículo de disseminção de patógenos (NG et al., 2018). Na Índia, já foi constatado a presença de *E. coli* resistente comprometendo a qualidade de peixes para consumo humano, reforçando o alerta em relação à saúde pública e a necessidade de vigilância constante sobre as vias de disseminação de patógenos resistentes (SIVARAMAN et al., 2020).

E. coli geralmente não causa doença nos peixes, mas podem expressar fatores de virulência e causar doença nos humanos. Desse modo, torna-se essencial a adoção de boas práticas de fabricação e programas de autocontrole nos sistemas produtivos (GREENLEES et al., 1998). *Escherichia coli* é um bastonete gram-negativo da família *Enterobacteriaceae*, anaeróbia facultativa, não esporulada, com temperatura ótima de crescimento entre 36 e 40ºC, capaz de formar biofilmes e ser comensal do trato intestinal de animais de sangue quente e répteis (TENAILLON et al., 2010).

É um micro-organismo utilizado como indicador de higiene com potencial de disseminação por alimentos, água e solo (JANG et al., 2017). Além das cepas comensais, também existem espécies patogênicas que causam morte de cerca de 2 milhões de pessoas a cada ano (JANG et al., 2017). Estas cepas podem ser divididas em sete grupos de acordo com seu mecanismo de virulência: enterotoxigênica (ETEC), enteroinvasiva (EIEC), enteropatogênica (EPEC) difusamente aderente (DAEC), aderente invasivo (AIEC), enteroagregativo (EAEC) e produtora de Shiga-toxina (STEC) (CROXEN et al., 2013).

No Brasil, o Ministério da Saúde relata que *E. coli* foi o agente mais envolvido em surtos alimentares reportados no seu último relatório, sendo associado ao consumo de diversos produtos de origem animal (MS, 2019), sendo que os riscos são agravados pelo hábito de consumo de produtos crus (WALKER et al., 2018). A bactéria possui diferentes mecanismo de patogenicidade, mas, via de regra, causa manifestações gastroentéricas como vômito e diarreia (PAITAN, 2018). Em virtude da interação desta com meio ambiente, saúde pública e animal, faz-se necessário uma abordagem integrativa de modo a avaliar impactos e riscos epidemiológicos (PAITAN, 2018).

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CAPÍTULO 2
Artigo Científico

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Article

Occurrence of antimicrobial resistant *Escherichia coli* in a fish production chain

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Abstract: The intensification of production has been a reality in aquaculture, which increases the density of creation, stress and leaves the animals more susceptible to diseases, increasing the risk of compromising the microbiological quality of the final product. One of the risks is the presence of zoonotic pathogens such as Escherichia coli, a microorganism associated with food outbreaks. In addition to the pathogenicity of microorganisms, the resistance of bacteria to antimicrobials is a worldwide concern, which compromises the treatment of human beings and reduces the possibilities of optimal therapy. Thus, the objective of this work was to characterize the resistance profile of E. coli isolates obtained from a tilapia production chain with representative samples of animal production, industrial environment and final product. E. coli was identified at all points collected, and from the 240 samples analyzed, 61 resistance profiles were found, 21 of which were multi-resistance. There was a 31% reduction in the presence of the pathogen along the production chain ($P< 0.0001$) when comparing post-bleed carcass and final product. The collection points referring to animal production showed a greater number of multiresistant strains, with isolates resistant to up to 8 antimicrobial bases. The research is a warning about the indiscriminate use of antimicrobials, the multidrug resistance present in animal production and the importance of epidemiological surveys,

monitoring and investigation regarding the risk to public, animal and environment health. It also reinforces the need for caution in the use of antimicrobials in production environments and incentives for the use of technologies as alternative means.

Keywords: antibiotics, microbial sensitivity, tilapia.

Introduction

Brazil is recognized worldwide for its agro-export potential, moving the country's economy and generating employment and income in the various work chains involved in agribusiness. Contributing to food security in several regions of the world, fish represents an important source of animal protein (FAO, 2020). The production of this food matrix is a growing market, with fish farming reaching 800,000 tons produced in 2020 in Brazil (PEIXE BR, 2021), evidencing the importance and growth of this chain and its economic and social impacts, since about 8% of the world population is dependent on this sector (FAO, 2018).

The production of tilapia (Oreochromis spp.) stands out worldwide. For Brazil, the species represents about 60% of the Brazilian crop production, keeping the country as the fourth largest producer in the world (PEIXE BR, 2021). The intensification of the system is a direct reflection of globalization, which has a growing demand for animal products (DAWOOD et al., 2016). This increase is increasingly associated with intensive aquaculture, with the aim of strengthening the productive and economic potential of this food matrix (REBELATO et al., 2015). As a result, the adoption of biosecurity measures and good agricultural practices are essential for the prevention of disease outbreaks that involve the chain and that may compromise consumer health due to their zoonotic nature (VAN BOECKEL et al., 2017).

Among the common pathogens between humans and animals, *E. coli* stands out. This bacterium has already been found in aquacultures associated with resistance to most antibiotics used in clinical medicine and is characterized by its efficiency in disseminating resistance genes (LIHAN et al., 2021). Thus, the objective of this work is to evaluate the resistance of *E. coli* from animals, industrial environment and final product of a tilapia production chain when subjected to phenotypic tests with different classes of antibiotics of relevance to human health, contemplating an approach in One Health.

Materials and methods

Ethical aspects

The research project was approved by the Ethics Committee for Research on Human Beings with Certificate of Presentation for Ethical Assessment (CAAE) 04536218.6.3006.5411 and opinion 3,489,847. Those involved were submitted to the research adherence through the Free and Informed Consent Term (ICF), collaborating in an autonomous, conscious, free and informed manner.

Sample collection

The project was carried out in a fish and fish products processing unit that works under the supervision of the State Inspection Service and included in the Brazilian System of Inspection of Products of Animal Origin (SISBI-POA). The collections were carried out in ten visits, so that in each visit a single origin of tilapia was evaluated in the different stages of slaughter and processing. Origins were identified from A to F and samples were collected at the following production points: carcass (K) (n= 100); scaling wastewater (AE) (n=10); filleting wastewater (AF) (n=10); fillet toilet water (AT) (n=10); fillet handling surface (n =10) (S); and pre-packaged fillets (FP) (n=100) (Table 1).

The surface collections of carcass and fillets were obtained by superficial rinsing in sterile bags containing 100 ml of sterile saline solution (0.85%). The carcasses were still collected in the reception

boxes from the farms and the fillets in boxes before being packed. The samples of equipment surfaces were obtained at different stages of processing being collected by superficial smear with sponges previously hydrated with 0.85% NaCl saline solution (m/v) and sterile. For this procedure, sterile molds measuring 100 cm2 (10 cm x 10 cm) were used to delimit the area to be sampled. At the points where the water was collected, sterile flasks containing sodium thiosulfate were used. After collection, the samples remained cooled in styrofoam with ice until they arrived at the Laboratory of Inspection and Technology of Products of Animal Origin at FAMEV-UFU, where they were analyzed for the presence of *E. coli* and, later, the sensitivity tests were performed. antimicrobial.

Table 1- Description of points, samples and collection methods carried out in the tilapia processing unit

Kind of sample	Collect point	Method	Area/ volume	n/visit	n total
Animal	Carcass after bleeding (K)	Rinse	100 mL	10	100
Environment	Filleting Water (AF)	Bottle	25 mL	1	10
Environment	Water from scaling (AE)	Bottle	25 mL	1	10
Environment	Water from toilet of fillets (AT	Bottle	25 mL	1	10
Environment	Surface of equipments (S)	Sponge	400 cm²	1	10
Environment	Fillet pre-packaged (FP)	Rinse	100 mL	10	100
Total				**24**	**240**

Isolation and characterization of *E. coli*

The isolation of *E. coli* was carried out by the conventional microbiological technique. The samples of carcass, environment, feces and products were processed by adding 250 mL of Buffered Peptone Water (PTA) and, later, they were homogenized and incubated at 37ºC for 18-24 h. After this period, they were incubated in *Escherichia coli* broth at 45ºC for 48h. Subsequently, aliquots of the broth were streaked onto MacConkey Agar and incubated at 37ºC for 18-24 h.

All the suspected colonies selected were submitted to biochemical tests by cultivating the strains in the culture media EPM (Escola Paulista de Medicina), MILi (motility, indole and lysine) and Simmons Citrate (TOLEDO, FONTES & TRABULSI, 1982a, 1982b). Samples biochemically confirmed as *E. coli* were stored on nutrient agar and in BHI (Brain Heart Infusion) broth added with 10% glycerol and kept frozen.

Characterization of the phenotypic profile of antibiotic resistance in *E. coli* isolates

The sensitivity of *E. coli* isolates to antimicrobial agents was evaluated using the Kirby-Bauer disk diffusion methodology (BAUER et al., 1966), according to international recommendations (CLSI, 2020; CLSI VET, 2018). Antimicrobials used in animal production and commonly used for human health were tested: amoxicillin - AMO (10 μg); ceftiofur - CTF (30 μg); aztreonam - ATM (30 μg); imipenem - IPM (10 μg); ciprofloxacin - CIP (5 μg); tetracycline - TET (30 μg); gentamicin - GEN (10 μg), sulfamethoxazole + trimethoprim - SUT (23.75/1.25 μg), chloramphenicol - CLO (30 μg) and azithromycin - AZI (15 μg). Extended spectrum lactamase (ESBL) two antimicrobials were used as screening, ceftazidime – CAZ (10 μg) and cefotaxime-CTX (5 μg), both third-generation cephalosporins (EUCAST, 2013).

E. coli isolates that showed increased zones of inhibition around any cephalosporin in the disc direction with amoxicillin with clavulanic acid were considered positive for ESBL enzyme production (EUCAST, 2013). *E. coli* isolates were considered sensitive or resistant according to the inhibition halo and *E. coli* isolates resistant to more than three classes of antimicrobials were defined as multidrug-resistant (MDR) (EMA, 2017).

Statistical Analysis

Descriptive statistics were used to characterize the resistance data, calculating the frequency of E. coli and resistant isolates at each point. Frequency results were compared by Chi-square and between collection points and collection days by Kruskal-Wallis using GraphPad Prisma 9.2.0 software (P<0.05).

Results

Of the 240 samples analyzed, 50.8% (122) were positive for *Escherichia coli*. All analyzed points had positive samples, with frequency ranging from 30 to 70% (Table 2). Among the positive samples, 403 isolates were biochemically confirmed as *E. coli*, and 221 strains were found in the post-bleeding carcass samples, 20 in the scaling water, 20 in the filleting water, 19 in the water from the toilet of the fillets, 11 on cut surfaces and 112 on pre-packaged fillets.

Table 2- Frequency of positivity for the presence of *Escherichia coli* in a tilapia processing unit in Minas Gerais-Brazil.

Collection points	n	Positive samples (%)	
		Escherichia coli	P value
Animal/Carcass			
Post bleed	100	66 (66,0)	<0,0001
Pre-packaged fillet	100	35 (35,0)	
Environment			
Scaling water	10	5 (50,0)	
Filleting water	10	6 (60,0)	
Toilet water	10	7 (70,0)	
Table surface	10	3 (30,0)	
Total	240	122 (50,8)	

*Chi-square test

A 31% reduction in the frequency of positivity for *E. coli* was observed between the initial point (post-bleed carcass) and the final point of collection (pre-package fillet) (P<0.0001). The same points, when analyzed from the perspective of visits made to the slaughterhouse, were divided and identified according to each origin (Table 03). It is worth mentioning that in different visits samples were collected from the same origin, such as visits 1 and 2 with origin A, 3 and 4 with origin B, 5 and 8 with origin C and 6 and 9 with origin D. 7 and 10 were from independent origins, different from the others. It is noted that visits 6 (origin D) and 8 (origin C), with 75% of the samples positive for the microorganism, had a higher frequency of positivity than visits 2 (origin A) and 9 (origin D) with 20 .8% (P<0.05).

Table 3- Frequency of positivity for the presence of *Escherichia coli* in a tilapia processing unit according to the visit carried out for collection

Visit	**Origin**	**n**	***Escherichia coli***	**%**
1	A	24	10	41,6[a,b]
2	A	24	5	20,8[b]
3	B	24	12	50,0 [a,b]
4	B	24	16	66,6 [a,b]
5	C	24	11	45,8 [a,b]
6	D	24	18	75,0[a]
7	E	24	16	66,6 [a,b]
8	C	24	18	75,0[a]
9	D	24	5	20,8[b]
10	F	24	11	45,8 [a,b]
			P value	<0,0001

In the antimicrobial susceptibility test, ten agents from nine different classes were selected for testing (Table 4). Of the 403 *E. coli* strains submitted to antibiotic sensitivity tests, 145 (36.0%) were classified as sensitive to all tested antimicrobials and 133 (33.0%) were resistant to at least two antimicrobials.

The highest frequencies of resistance presented by the isolates were amoxicillin (35.73%), tetracycline (30.77%), ciprofloxacin (26.30%), association of sulfonamide with trimethoprim (12.41%) and chloramphenicol (11, 66%), respectively. Among the greatest resistances found in the present study, there is the resistance of the bacteria to chloramphenicol (11.66%). It was observed that the pathogen was associated with a lower resistance to gentamicin (1.99%), azithromycin (2.73%), ceftiofur (2.98%), aztreonam (4.71%) and imipenem (6.95 %) (Table 04).

Table 4- Antimicrobial resistance of *Escherichia coli* isolates obtained at different stages of slaughter and processing of tilapia in Minas Gerais-Brazil.

Class	Antibiotic	Resistence of isolates (n=403)	
		n	(%)
Aminoglycosides	Gentamicin	8	1,99
Beta-lactams	Amoxicilin	144	35,73
	Aztreonam	19	4,71
Carbapenems	Imipenem	28	6,95
Cephalosporins 3ªg	Ceftiofur	12	2,98
Amphenicols	Chloramphenicol	47	11,66
Fluorquinolones	Ciprofloxacin	106	26,3
Macrolides	Azithromycin	11	2,73
Sulfonamides	trimethoprim-sulfamethoxazole	50	12,41
Tetracyclines	Tetracycline	124	30,77

Considering the results of resistance to the antimicrobials tested, it was possible to identify 47 multiresistant *E. coli* isolates (MDR) (47/403), from 31 samples analyzed (Table 5). In this evaluation, it was possible to show that visit 8 (origin C) had a higher frequency of multidrug resistance than visits 1, 2, 3, 4, 7 and 9. In addition, it can be seen that the two highest frequencies of samples with isolates *E coli* MDR were identified in visits where animals of the same origin were being slaughtered, namely: visit 8 (41.6% of samples with MDR isolates) and visit 5 (20.8%), both from C.

It was also observed that, although *E. coli* was identified at all collection points, MDR isolates were not found at the AT and S points. It is also noted that 68.1% (32/47) of these multidrug-resistant bacteria were identified at points K and AE, which are representative of animal production.

Table 5- Distribution of positive samples for multidrug resistant *E. coli* and number of isolates obtained according to point of collection, visit and origin

Visit	Origin	Samples MDR positive	Isolates MDR per collection point - n (%)					
		n (%)	K	AE	AF	AT	S	FP
1	A	2 (8,3)[b]	0	0	2 (4,26)	0	0	0
2	A	1 (4,1)[b]	1 (2,13)	0	0	0	0	0
3	B	2 (8,3)[b]	0	0	0	0	0	2 (4,26)
4	B	1 (4,1)[b]	1 (2,13)	0	0	0	0	0
5	C	5 (20,8)[a,b]	9 (19,15)	4 (8,51)	0	0	0	0
6	D	5 (20,8)[a,b]	1 (2,13)	0	2 (4,26)	0	0	6 (12,77)
7	E	1 (4,1)[b]	1 (2,13)	0	0	0	0	0
8	C	10 (41,6)[a]	8 (17,02)	3 (6,38)	0	0	0	2 (4,26)
9	D	0[b]	0	0	0	0	0	0
10	F	4 (16,6)[a,b]	4 (8,51)	0	1 (2,13)	0	0	0
	Valor de P	<0,0001						

*n – number of multidrug-resistant isolates per collection point; *K = post-bleed carcass; *AE = scaling water; *AF – filleting water; *AT – fillet toilet water; *S = handling surface; *FP = pre-packaged fillets. Kruskal-Wallis test, Dunn's multiple comparison test.

Analyzing the variations between the resistance results presented by each isolate, 61 profiles were identified, of which 80% were found at point K, 16.5% at point AE, 11.5% at point AF, 9.8% at point AT, 3.3% at point S and 31.2% at point FP. Of these, 21 profiles were considered to be of multidrug resistance and are shown in table 6. It is noteworthy that the isolates with the highest resistance profile were identified in samples from points related to the origin of the animals (AE and K), with one isolate being resistant to 8 antimicrobials, two isolates resistant to 7, and two isolates resistant to 6 antimicrobials.

Table 6- Multidrug resistance profiles of multidrug resistant *E. coli* isolates and sample origin

Number of isolate	Resistence simultaneous		Profile resistence	Origin
	Class antimicrobial	Antimicrobial agents		
1	8	8	TET-CIP-SUT-CLO-AZI-CTF-IPM-ATM	Scaling water
1	7	7	AMO-TET-CIP-SUT-CLO-CTF-IPM	Scaling water
1	6	7	AMO-TET-CIP-SUT-CLO-IPM-ATM	Post-bleed carcass
1	6	6	AMO-TET-CIP-SUT-CLO-GEN	Post-bleed carcass
1	6	6	AMO-TET-CIP-SUT-IPM-GEN	Scaling water
8	5	5	AMO-TET-CIP-SUT-CLO	Post-bleed carcass, Scaling water, Fillets
2	5	5	AMO-TET-CIP-SUT-IPM	Post-bleed carcass, Scaling water
1	5	5	AMO-TET-CIP-SUT-AZI	Post-bleed carcass
1	4	5	AMO-CLO-CTF-IPM-ATM	Post-bleed carcass
1	5	5	AMO-CIP-SUT-CTF-IPM	Filleting water
1	5	5	AMO-TET-CIP-CLO-GEN	Post-bleed carcass
7	4	4	AMO-TET-CIP-SUT	Post-bleed carcass, Fillets
6	4	4	AMO-TET-CIP-CLO	Post-bleed carcass, Filleting water, Fillets
6	4	4	TET-CIP-SUT-CLO	Post-bleed carcass, Filleting water, Fillets
2	3	4	AMO-TET-CIP-ATM	Fillets
2	4	4	AMO-TET-SUT-AZI	Post-bleed carcass
1	4	4	AMO-TET-SUT-CLO	Filleting water
1	4	4	AMO-CIP-IPM-GEN	Post-bleed carcass
1	4	4	AMO-TET-SUT-IPM	Post-bleed carcass
1	4	4	TET-AZI-CTF-ATM	Fillets
1	4	4	CIP-CLO-CTF-IPM	Post-bleed carcass

TET-Tetracycline; CIP-Ciprofloxacin; SUT-Sulfamethoxazole and trimethoprim; CLO-Chloramphenicol; AZI-Azithromycin; CTF-Ceftiofur; IPM-Imipenem; ATM-Aztreonam; AMO-Amoxicillin; GEN-Gentamycin;

Discussion

A reduction in the frequency of positivity for *E. coli* was observed between the initial point (post-bleeding carcass) and the final collection point (pre-package fillet) (P<0.0001). This shows that despite the presence of the agent, there is a reduction throughout production, which suggests that the industry's self-control programs have been efficient in reducing biological hazards. In this context, it is important to emphasize the role of the government as a supervisory agent and of companies as responsible for acquiring quality raw materials and always seeking a final product that is safe for the consumer (LUPIEN, 2007; DE FILIPPIS et al., 2018).

The results showed that the frequency of positivity for *E. coli* varied according to the day of collection, however, there were antagonistic results when comparing the same origin as indicated by the difference between collections 6 and 9, with tilapia from the same production origin (P<0.05). It is noted that visits 6 (origin D) and 8 (origin C), with 75% of the samples positive for the microorganism, had a higher frequency of positivity than visits 2 (origin A) and 9 (origin D) with 20 .8% (P<0.05), showing that the origin is not exclusively responsible for positivity for *Escherichia coli*. This may indicate that each batch has a specific microbiome, with different responses when challenged in relation to sanitary conditions; management during the harvesting of the property, for example; and environmental factors, affecting the dynamic presence and distribution of pathogens (MIRANDA et al., 2013).

Of the 403 strains of *E. coli* submitted to antibiotic sensitivity tests, 145 (36.0%) were classified as sensitive to all tested antimicrobials and 133 (33.0%) were resistant to at least two antimicrobials, being classified as extensively resistant (JEONG et al., 2007). Results of resistance to tetracycline (38%) and sulfa-trimethoprim (12%) were found by Jiao (2007) and are close to those found in this work, as well as resistance to ciprofloxacin (22%) found in the work of Boss (2016), both in fish.

In addition to these data, other studies are also concerned about the resistance found in relation to amoxicillin and tetracycline, which is a risk for the environment and future generations due to its widespread misuse, with high rates of resistance and low efficacy (WEIR et al. ., 2012). Resistance to tetracycline is considered frequent in most aquatic productions due to its wide use and corroborates what was mentioned above (TUSELVJAK et al., 2013). Results consistent with the present study have already been found in relation to the susceptibility of *E. coli* isolates to gentamicin and aztreonam, since they are less frequently used antibiotics in aquaculture (ROCHA et al., 2014).

Among the greatest resistances found in the present study, there is the resistance of the bacteria to chloramphenicol (11.66%). This pharmacological base has been banned in animal production since 2003 because its residues constitute a risk to public health (BRASIL, 2003). However, the literature suggests that this drug is used even after its legal ban (PACHECO-SILVA et al., 2014). Therefore, in addition to the possibility of a horizontal transmission of resistance genes (RICHARDSON et al., 2018), a possible illegal use of this antimicrobial inducing resistance in the analyzed microbiome should be taken into account (MILLER et al., 2018; SMITH et al., 2018; SMITH et al., 2018). al., 1993).

Resistant bacteria are considered one of the greatest challenges to human and veterinary medicine today. In aquaculture, they have been associated with findings of residues in the aquatic environment and alteration of the local microbiome, contaminating fish and increasing the risk of resistant pathogens reaching humans (MILLANAO et al., 2018). The main problem about microbial resistance when they reach pathogens that affect humans is the limitation of possible therapies and/or the efficiency of pharmacological bases (HEUER et al., 2009).

It is a reality in Brazil, as already reported in Europe by Brown (1987), the worrying ease of acquiring a variety of pharmacological bases that are used in animal production and, in many cases, without a medical-veterinary indication. In this context, there is a need to implement legislation that determines and controls the use and commercialization of medicines for veterinary use in a more

austere way.

It was also observed that, although *E. coli* was identified at all collection points, MDR isolates were not found in the water points of the fillet toilet and cut surfaces (AT and S). It is also noted that 68.1% (32/47) of these multidrug-resistant bacteria were identified at points K and AE, which are representative of animal production, reflecting the neglected and empirical use that occurs on rural properties. This corroborates the fact that the indiscriminate use of antimicrobials in animal production is a concern and requires immediate changes, reinforcing international recommendations on surveillance and monitoring programs by competent authorities around the world (SMITH et al., 2013). Evidence of the problem is given by the association of intensive production systems with a greater possibility of emergence of resistance when compared to animals in the natural environment (JACKSON et al., 2020).

Although there is a reduction both in the frequency of *E. coli* positivity and in the amount of MDR positive samples observed along the production line, the high number of resistance profiles found in animal production is remarkable. This is a reality that evidences the indiscriminate use of antimicrobials in animal production that amplifies the chance of selection of resistant bacteria (WENCEWICZ, 2019). A major challenge in addition to the occurrence of multidrug resistance of isolates is the possibility of reaching humans through the food chain, requiring a broad approach that directs the use, with adequate and assisted indication in all links of the animal chain (DE ALCANTARA RODRIGUES et al. , 2020; MANYI-LOH et al., 2018).

The occurrence of different resistance profiles found in different origins suggests that when animals are submitted to a challenge, they may respond differently when using the same therapy, precisely because of the variation in the local microbiome and, therefore, causing a difference between the profiles. of resistance from each location (HO et al., 2000). The profiles that showed a greater amount of ineffective antimicrobial bases are from points related to animal production, being evidence that there is a bottleneck in animal production, which corroborates the selection of resistant strains that may lead to failures in therapies used in human health. and animal, in addition to ecosystem modification (ISLAM et al., 2019).

The present study did not identify any extended-spectrum beta-lactamase-producing strain, unlike previous studies such as the one by Sivaraman (2020). One possibility for this difference in result is the characteristic of local production exerting less selective pressure on the microbiome. Furthermore, the absence of the enzyme production phenotype does not rule out the possibility of strains presenting the gene and transmitting it to other bacteria present in the medium and, consequently, the risk to public health. However, a molecular analysis to answer this gap was not performed in this research.

The damage caused by years of indiscriminate use of antibiotics cannot be undone, but there are already alternatives that can minimize their use and, consequently, collaborate to reduce consumption and selective pressure caused by antimicrobials. The use of herbal medicines appears as a natural alternative, non-aggressive to the environment and with antimicrobial properties (VALLADÃO et al., 2015). Another strategy is the use of essential oils in the prevention and treatment of diseases in fish, contributing to the reduction of the use of antibiotics (DA CUNHA et al., 2018).

The use of vaccines for disease prevention is booming and is already an option that helps to reduce the use of antimicrobials and contributes significantly to animal health in intensive productions (HASTEIN et al., 2005). Among the manufacturing technologies are the production of vaccines by inactivated pathogens, genetic recombination against bacterial and viral agents, tools that have already been shown to be an important variable for the success of the production of fish species (SOMMERSET et al., 2005) and also the application of nanotechnology for disease management (SHAALAN et al., 2016; SABO-ATTWOOD et al., 2021).

The improvement of biosecurity is a basic and extremely important tool to minimize the impacts of antimicrobial use. In this context, good agricultural practices, water management, adequate cleaning, adequate diagnosis of diseases that affect the herd and improvement in infrastructure are included (HENRIKSSON et al., 2018). Finally, probiotics are also an alternative to the use of antimicrobials, since they have an influence on water quality, increase the immune response and antiviral effects (BALCÁZAR et al., 2006).

Conclusion

E. coli is present in the production chain of tilapia, being found in all analyzed points and with variable profiles of microbial resistance. In addition, production-related samples were the main source of multidrug-resistant strains and a possible relationship with the origin of the animals. The study raises the alarm about the risk to public health, animal health and the environment, reinforcing the importance of care with the production system since in the case of aquaculture there is an even greater potential for the dissemination of resistance through water. It is also necessary to reinforce the need to restrict the use of antimicrobials in animal production and encourage the use of technologies that can be used as alternative means.

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- *Case reports:* Case reports present detailed information on the symptoms, signs, diagnosis, treatment (including all types of interventions), and outcomes of an individual patient. Case reports usually describe new or uncommon conditions that serve to enhance medical care or highlight diagnostic approaches.

Submission Process

Manuscripts for *Veterinary Sciences* should be submitted online at **susy.mdpi.com**. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the **criteria to qualify for authorship**) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the **submission website**. Once you have registered, **click here to go to the submission form for *Veterinary Sciences***. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Accepted File Formats

Authors must use the **Microsoft Word template** or **LaTeX template** to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the Editorial Office **vetsci@mdpi.com**. Accepted file formats are:

- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, the ***Veterinary Sciences* Microsoft Word template file** must be used. Please insert your graphics (schemes, figures, *etc.*) in the main text after the paragraph of its first citation.

- *LaTeX:* Manuscripts prepared in LaTeX must be collated into one ZIP folder (including all source files and images, so that the Editorial Office can recompile the submitted PDF). When preparing manuscripts in LaTeX, please use the ***Veterinary Sciences* LaTeX template files**. You can now also use the online application **writeLaTeX** to submit articles directly to *Veterinary Sciences*. The MDPI LaTeX template file should be selected from the **writeLaTeX template gallery**.

- *Supplementary files:* May be any format, but it is recommended that you use common, non-proprietary formats where possible (see **below** for further details).

Disclaimer: Usage of these templates is exclusively intended for submission to the journal for peer-review, and strictly limited to this purpose and it cannot be used for posting online on preprint servers or other websites.

Free Format Submission

Veterinary Sciences now accepts free format submission:

- We do not have strict formatting requirements, but all manuscripts must contain the required sections: Author Information, Abstract, Keywords, Introduction, Materials & Methods, Results, Conclusions, Figures and Tables with Captions, Funding Information, Author Contributions, Conflict of Interest and other Ethics Statements. Check the Journal ***Instructions for Authors*** for more details.

- Your references may be in any style, provided that you use the consistent formatting throughout. It is essential to include author(s) name(s), journal or book title, article or chapter title (where required), year of publication, volume and issue (where appropriate) and pagination. DOI numbers (Digital Object Identifier) are not mandatory but highly encouraged. The bibliography software package *EndNote*, ***Zotero***, *Mendeley*, *Reference Manager* are recommended.

- When your manuscript reaches the revision stage, you will be requested to format the manuscript according to the journal guidelines.

Cover Letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work. It should explain why the manuscript fits the scope of the journal.

Any prior submissions of the manuscript to MDPI journals must be acknowledged. If this is the case, it is strongly recommended that the previous manuscript ID is provided in the submission system, which will ease your current submission process. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

All cover letters are required to include the statements:

- We confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal.

- All authors have approved the manuscript and agree with its submission to (journal name).

Author Biography

Authors are encouraged to add a biography (maximum 150 words) to the submission and publish it. This should be a single paragraph and should contain the following points:

1. Authors' full names followed by current positions;
2. Education background including institution information and year of graduation (type and level of degree received);
3. Work experience;
4. Current and previous research interests;
5. Memberships of professional societies and awards received.

Note for Authors Funded by the National Institutes of Health (NIH)

This journal automatically deposits papers to PubMed Central after publication of an issue. Authors do not need to separately submit their papers through the NIH Manuscript Submission System (NIHMS, **http://nihms.nih.gov/**).

Manuscript Preparation

General Considerations

- **Research manuscripts** should comprise:
    - **Front matter**: Title, Author list, Affiliations, Abstract, Keywords
    - **Research manuscript sections**: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).

- **Back matter**: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, **References**.

- **Review manuscripts** should comprise the **front matter**, literature review sections and the **back matter**. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the **PRISMA** guidelines.

- **Case reports** should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.

- **Graphical Abstract:**

  A graphical abstract (GA) is an image that appears alongside the text abstract in the Table of Contents. In addition to summarizing the content, it should represent the topic of the article in an attention-grabbing way. Moreover, it should not be exactly the same as the Figure in the paper or just a simple superposition of several subfigures. Note that the GA must be original and unpublished artwork. Any postage stamps, currency from any country, or trademarked items should not be included in it.

  The GA should be a high-quality illustration or diagram in any of the following formats: PNG, JPEG, TIFF, or SVG. Written text in a GA should be clear and easy to read, using one of the following fonts: Times, Arial, Courier, Helvetica, Ubuntu or Calibri.

  The minimum required size for the GA is 560 × 1100 pixels (height × width). The size should be of high quality in order to reproduce well.

- **Acronyms/Abbreviations/Initialisms** should be defined the first time they appear in each of three sections: the abstract; the main text; the first figure or table. When defined for the first time, the acronym/abbreviation/initialism should be added in parentheses after the written-out form.

- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible.

- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on **Deposition of Sequences and of Expression Data**.

- **Equations:** If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.

- **Research Data and supplementary materials:** Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Disclose at the submission stage any restrictions on the availability of materials or information. Read the information about **Supplementary Materials** and Data Deposit for additional guidelines.

- **Preregistration:** Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.

- **Guidelines and standards:** MDPI follows standards and guidelines for certain types of research. See **https://www.mdpi.com/editorial_process** for further information.

Front Matter

These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.

- **Author List and Affiliations:** Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, and country. At least one author should be designated as corresponding author, and his or her email address and other details should be included at the end of the affiliation section. Please read the **criteria to qualify for authorship**.

- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.

- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.

- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.

- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.

- **Conclusions:**This section is not mandatory but can be added to the manuscript if the discussion is unusually long or complex.

- **Patents:** This section is not mandatory but may be added if there are patents resulting from the work reported in this manuscript.

Back Matter

- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.

- **Funding:** All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.
Please add: “This research received no external funding” or “This research was funded by [name of funder] grant number [xxx]” and “The APC was funded by [XXX]” in this section. Check carefully that the details given are accurate and use the standard spelling of funding agency names at https://search.crossref.org/funding, any errors may affect your future funding.

- **Acknowledgments:** In this section you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

- **Author Contributions:** Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author’s contribution to the study); AND agrees to be personally accountable for the author’s own contributions and for ensuring that questions related to the

accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.", please turn to the **CRediT taxonomy** for the term explanation. For more background on CRediT, see **here**. "**Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully**".

- **Institutional Review Board Statement:** In this section, please add the Institutional Review Board Statement and approval number for studies involving humans or animals. Please note that the Editorial Office might ask you for further information. Please add "The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of NAME OF INSTITUTE (protocol code XXX and date of approval)." OR "Ethical review and approval were waived for this study, due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans or animals. You might also choose to exclude this statement if the study did not involve humans or animals.

- **Informed Consent Statement:** Any research article describing a study involving humans should contain this statement. Please add "Informed consent was obtained from all subjects involved in the study." OR "Patient consent was waived due to REASON (please provide a detailed justification)." OR "Not applicable" for studies not involving humans. You might also choose to exclude this statement if the study did not involve humans. Written informed consent for publication must be obtained from participating patients who can be identified (including by the patients themselves). Please state "Written informed consent has been obtained from the patient(s) to publish this paper" if applicable.

- **Data Availability Statement:** In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Please refer to suggested Data Availability Statements in section "**MDPI Research Data Policies**". You might choose to exclude this statement if the study did not report any data.

- **Conflicts of Interest:** Authors must identify and declare any personal circumstances or interest that may be perceived as influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. *Veterinary Sciences* does not publish studies funded partially or fully by the tobacco industry. Any projects funded by industry must pay special attention to the full declaration of funder involvement. If there is no role, please state "The sponsors had no role in the design, execution, interpretation, or writing of the study". For more details please see **Conflict of Interest**.

- **References:** References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as **EndNote**, **ReferenceManager** or **Zotero** to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. If available online, you may use reference style 9. below.

- Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for **Endnote** and **Zotero** are available.

References should be described as follows, depending on the type of work:

Journal Articles:
1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.
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2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196.
3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher

Location, Country, Year; Volume 3, pp. 154–196.
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Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as **WebCite**. Archived websites should be cited using the link provided as follows:
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See the **Reference List and Citations Guide** for more detailed information.

Preparing Figures, Schemes and Tables

- File for Figures and Schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.
- *Veterinary Sciences* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.
- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).
- All Figures, Schemes and Tables should have a short explanatory title and caption.
- All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.
- Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

Original Images for Blots and Gels Requirements

For the main text, please ensure that:

- All experimental samples and controls used for one comparative analysis are run on the same blot/gel.
- Image processing methods, such as adjusting the brightness or contrast, do not alter or distort the information in the figure and are applied to every pixel. High-contrast blots/gels are discouraged.
- Cropped blots/gels present in the main text retain all important information and bands.
- You have checked figures for duplications and ensured the figure legends are clear and accurate. Please include all relevant information in the figure legends and clearly indicate any re-arrangement of lanes.

In order to ensure the integrity and scientific validity of blots (including, but not limited to, Western blots) and the reporting of gel data, original, uncropped and unadjusted images should be uploaded as Supporting Information files at the time of initial submission.

A single PDF file or a zip folder including all the original images reported in the main figure and supplemental figures should be prepared. Authors should annotate each original image, corresponding to the figure in the main article or

supplementary materials, and label each lane or loading order. All experimental samples and controls used for one comparative analysis should be run on the same blot/gel image. For quantitative analyses, please provide the blots/gels for each independent biological replicate used in the analysis.

[Return to top]

Supplementary Materials, Data Deposit and Software Source Code

MDPI Research Data Policies

MDPI is committed to supporting open scientific exchange and enabling our authors to achieve best practices in sharing and archiving research data. We encourage all authors of articles published in MDPI journals to share their research data. Individual journal guidelines can be found at the journal 'Instructions for Authors' page. Data sharing policies concern the minimal dataset that supports the central findings of a published study. Generated data should be publicly available and cited in accordance with journal guidelines.

MDPI data policies are informed by **TOP Guidelines** and **FAIR Principles**.

Where ethical, legal or privacy issues are present, data should not be shared. The authors should make any limitations clear in the Data Availability Statement upon submission. Authors should ensure that data shared are in accordance with consent provided by participants on the use of confidential data.

Data Availability Statements provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

Below are suggested Data Availability Statements:

- Data available in a publicly accessible repository
  The data presented in this study are openly available in [repository name e.g., FigShare] at [**doi**], reference number [reference number].

- Data available in a publicly accessible repository that does not issue DOIs
  Publicly available datasets were analyzed in this study. This data can be found here: [link/accession number]

- Data available on request due to restrictions eg privacy or ethical
  The data presented in this study are available on request from the corresponding author. The data are not publicly available due to [insert reason here]

- 3rd Party Data
  Restrictions apply to the availability of these data. Data was obtained from [third party] and are available [from the authors / at URL] with the permission of [third party].

- Data sharing not applicable
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- Data is contained within the article or supplementary material
  The data presented in this study are available in [insert article or supplementary material here]

Data citation:

- [dataset] Authors. Year. Dataset title; Data repository or archive; Version (if any); Persistent identifier (e.g., DOI).

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository such as **GitHub** or uploading as supplementary information to the publication. The name, version, corporation and location information for all software used should be clearly indicated. Please include all the parameters used to run software/programs analyses.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable; however, we recommend that common, non-proprietary formats are used where possible.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult **databib.org** or **re3data.org**. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal **Data** also accepts submissions of data set papers.

Deposition of Sequences and of Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited in one of the following databases: **GenBank**, **EMBL**, or **DDBJ**. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the **GEO database** or in the NCBI's **Sequence Read Archive (SRA)**.
- *New microarray data* must be deposited either in the **GEO** or the **ArrayExpress** databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool **SPIN**). Annotated protein structure and its reference sequence must be submitted to **RCSB of Protein Data Bank**.

All sequence names and the accession numbers provided by the databases must be provided in the Materials and Methods section of the article.

Deposition of Proteomics Data

Methods used to generate the proteomics data should be described in detail and we encourage authors to adhere to the "**Minimum Information About a Proteomics Experiment**". All generated mass spectrometry raw data must be deposited in the appropriate public database such as **ProteomeXchange**, **PRIDE** or **jPOST**. At the time of submission, please include all relevant information in the materials and methods section, such as repository where the data was submitted and link, data set identifier, username and password needed to access the data.

[Return to top]

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (**https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/**), revised in 2013. According to point 23 of this declaration, an approval from the local institutional review board (IRB) or other appropriate ethics committee must be obtained before undertaking the research to confirm the study meets national and international guidelines. As a minimum, a statement including the project identification code, date of approval, and name of the ethics committee or institutional review board must be stated in Section 'Institutional Review Board Statement' of the article.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study. If ethical approval is not required, authors must either provide an exemption from the ethics committee or are encouraged to cite the local or national legislation that indicates ethics approval is not required for this type of study. Where a study has been granted exemption, the name of the ethics committee which provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation regarding why ethical approval was not required.

A written informed consent for publication must be obtained from participating patients. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent for publication from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions. A **template permission form** is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission. Editors reserve the right to reject any submission that does not meet these requirements.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

If the study reports research involving vulnerable groups, an additional check may be performed. The submitted manuscript will be scrutinized by the editorial office and upon request, documentary evidence (blank consent forms and any related discussion documents from the ethics board) must be supplied. Additionally, when studies describe groups by race, ethnicity, gender, disability, disease, etc., explanation regarding why such categorization was needed must be clearly stated in the article.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs [1]':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Authors must include details on housing, husbandry and pain management in their manuscript.

For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [2], American Association for Laboratory Animal Science [3] or European Animal Research Association [4].

If national legislation requires it, studies involving vertebrates or higher invertebrates must only be carried out after obtaining approval from the appropriate ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be stated in Section 'Institutional Review Board Statement'. Research procedures must be carried out in accordance with national and institutional regulations. Statements on animal welfare should confirm that the study complied with all relevant legislation. Clinical studies involving animals and interventions outside of routine care require ethics committee oversight as per the American Veterinary Medical Association. If the study involved client-owned animals, informed client consent must be obtained and certified in the manuscript report of the research. Owners must be fully informed if there are any risks associated with the procedures and that the research will be published. If available, a high standard of veterinary care must be provided. Authors are responsible for correctness of the statements provided in the manuscript.

If ethical approval is not required by national laws, authors must provide an exemption from the ethics committee, if one is available. Where a study has been granted exemption, the name of the ethics committee that provided this should be stated in Section 'Institutional Review Board Statement' with a full explanation on why the ethical approval was not required.

If no animal ethics committee is available to review applications, authors should be aware that the ethics of their research will be evaluated by reviewers and editors. Authors should provide a statement justifying the work from an ethical perspective, using the same utilitarian framework that is used by ethics committees. Authors may be asked to provide this even if they have received ethical approval.

MDPI endorses the ARRIVE guidelines (**arriveguidelines.org/**) for reporting experiments using live animals. Authors and reviewers must use the ARRIVE guidelines as a checklist, which can be found at **https://arriveguidelines.org/sites/arrive/files/documents/ARRIVE%20Compliance%20Questionnaire.pdf**. Editors reserve the right to ask for the checklist and to reject submissions that do not adhere to these guidelines, to reject submissions based on ethical or animal welfare concerns or if the procedure described does not appear to be justified by the value of the work presented.

1. NSW Department of Primary Industries and Animal Research Review Panel. Three Rs. Available online: **https://www.animalethics.org.au/three-rs**
2. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes. Available online: **https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/388535/CoPanimalsWeb.pdf**
3. American Association for Laboratory Animal Science. The Scientific Basis for Regulation of Animal Care and Use. Available online: **https://www.aalas.org/about-aalas/position-papers/scientific-basis-for-regulation-of-animal-care-and-use**
4. European Animal Research Association. EU regulations on animal research. Available online: **https://www.eara.eu/animal-research-law**

Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the **Convention on Biological Diversity** and the **Convention on the Trade in Endangered Species of Wild Fauna and Flora**.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oryza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

Clinical Trials Registration

Registration

MDPI follows the International Committee of Medical Journal Editors (ICMJE) **guidelines** which require and recommend registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication.

Purely observational studies do not require registration. A clinical trial not only refers to studies that take place in a hospital or involve pharmaceuticals, but also refer to all studies which involve participant randomization and group classification in the context of the intervention under assessment.

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register and cite a reference to the registration in the Methods section. Suitable databases include **clinicaltrials.gov**, **the EU Clinical Trials Register** and those listed by the World Health Organisation **International Clinical Trials Registry Platform**.

Approval to conduct a study from an independent local, regional, or national review body is not equivalent to prospective clinical trial registration. MDPI reserves the right to decline any paper without trial registration for further peer-review. However, if the study protocol has been published before the enrolment, the registration can be waived with correct citation of the published protocol.

CONSORT Statement

MDPI requires a completed CONSORT 2010 **checklist** and **flow diagram** as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (**http://www.consort-statement.org**) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist.

[Return to top]

Sex and Gender in Research

We encourage our authors to follow the **'Sex and Gender Equity in Research – SAGER – guidelines'** and to include sex and gender considerations where relevant. Authors should use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Article titles and/or abstracts should indicate clearly what sex(es) the study applies to. Authors should also describe in the background, whether sex and/or gender differences may be expected; report how sex and/or gender were accounted for in the design of the study; provide disaggregated data by sex and/or gender, where appropriate; and discuss respective results. If a sex and/or gender analysis was not conducted, the rationale should be given in the Discussion. We suggest that our authors consult the full **guidelines** before submission.

[Return to top]

Borders and Territories

Potential disputes over borders and territories may have particular relevance for authors in describing their research or in an author or editor correspondence address, and should be respected. Content decisions are an editorial matter and where there is a potential or perceived dispute or complaint, the editorial team will attempt to find a resolution that satisfies parties involved.

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Publication Ethics Statement

Veterinary Sciences is a member of the Committee on Publication Ethics (**COPE**). We fully adhere to its **Code of Conduct** and to its **Best Practice Guidelines**.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Veterinary Sciences* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Veterinary Sciences* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.

- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- The journal accepts exact translations of previously published work. All submissions of translations must conform with our **policies on translations**.
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our **policy regarding Updating Published Papers**.
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the **Rights and Permissions** page.
- Plagiarism, data fabrication and image manipulation are not tolerated.
    - **Plagiarism is not acceptable** in *Veterinary Sciences* submissions.

      Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

      Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

      If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, we may publish a correction or retract the paper.
    - **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

      Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

      If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

  Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

Citation Policy

Authors should ensure that where material is taken from other sources (including their own published writing) the source is clearly cited and that where appropriate permission is obtained.

Authors should not engage in excessive self-citation of their own work.

Authors should not copy references from other publications if they have not read the cited work.

Authors should not preferentially cite their own or their friends', peers', or institution's publications.

Authors should not cite advertisements or advertorial material.

In accordance with COPE guidelines, we expect that "original wording taken directly from publications by other researchers should appear in quotation marks with the appropriate citations." This condition also applies to an author's own work. COPE have produced a discussion document on **citation manipulation** with recommendations for best practice.

[Return to top]

Reviewer Suggestions

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

[Return to top]

English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found **here**.

If you are not a native English speaker, we recommend that you have your manuscript professionally edited before submission or read by a native English-speaking colleague. This can be carried out by MDPI's **English editing service**. Professional editing will enable reviewers and future readers to more easily read and assess the content of submitted manuscripts. All accepted manuscripts undergo language editing, however **an additional fee will be charged** to authors if very extensive English corrections must be made by the Editorial Office: pricing is according to the service **here**.

[Return to top]

Preprints and Conference Papers

Veterinary Sciences accepts submissions that have previously been made available as preprints provided that they have not undergone peer review. A preprint is a draft version of a paper made available online before submission to a journal.

MDPI operates ***Preprints***, a preprint server to which submitted papers can be uploaded directly after completing journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. Check the *Preprints* **instructions for authors** for further information.

Expanded and high-quality conference papers can be considered as articles if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper. *Veterinary Sciences* does not publish pilot studies or studies with inadequate statistical power.

Unpublished conference papers that do not meet the above conditions are recommended to be submitted to the **Proceedings Series journals**.

[Return to top]

Authorship

MDPI follows the International Committee of Medical Journal Editors (**ICMJE**) guidelines which state that, in order to qualify for authorship of a manuscript, the following criteria should be observed:

- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgments. More detailed guidance on authorship is given by the **International Council of Medical Journal Editors (ICMJE)**.

Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication. We reserve the right to request confirmation that all authors meet the authorship conditions.

For more details about authorship please check **MDPI ethics website**.

Reviewers Recommendation

Authors can recommend potential reviewers. Journal editors will check to make sure there are no conflicts of interest before contacting those reviewers, and will not consider those with competing interests. Reviewers are asked to declare any conflicts of interest. Authors can also enter the names of potential peer reviewers they wish to exclude from consideration in the peer review of their manuscript, during the initial submission progress. The editorial team will respect these requests so long as this does not interfere with the objective and thorough assessment of the submission.

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Editorial independence is of utmost importance and MDPI does not interfere with editorial decisions. All articles published by MDPI are peer reviewed and assessed by our independent editorial boards, and MDPI staff are not involved in decisions to accept manuscripts. When making an editorial decision, we expect the academic editor to make their decision based only upon:

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- Adequacy of reviewer comments and author response;
- Overall scientific quality of the paper.

In all of our journals, in every aspect of operation, MDPI policies are informed by the mission to make science and research findings open and accessible as widely and rapidly as possible.

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Editorial staff or editors shall not be involved in processing their own academic work. Submissions authored by editorial staff/editors will be assigned to at least two independent outside reviewers. Decisions will be made by other Editorial Board Members who do not have a conflict of interest with the author. Journal staff are not involved in the processing of their own work submitted to any MDPI journals.

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According to The International Committee of Medical Journal Editors, “Authors should avoid entering into agreements with study sponsors, both for-profit and non-profit, that interfere with authors’ access to all of the study’s data or that interfere with their ability to analyze and interpret the data and to prepare and publish manuscripts independently when and where they choose.”

All authors must disclose all relationships or interests that could inappropriately influence or bias their work. Examples of potential conflicts of interest include but are not limited to financial interests (such as membership, employment, consultancies, stocks/shares ownership, honoraria, grants or other funding, paid expert testimonies and patent-licensing arrangements) and non-financial interests (such as personal or professional relationships, affiliations, personal beliefs).

Authors can disclose potential conflicts of interest via the online submission system during the submission process. Declarations regarding conflicts of interest can also be collected via the **MDPI disclosure form**. The corresponding author must include a summary statement in the manuscript in a separate section “Conflicts of Interest” placed just before the reference list. The statement should reflect all the collected potential conflict of interest disclosures in the form.

See below for examples of disclosures:

Conflicts of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stocks in Company Y. Author C has been involved as a consultant and expert witness in Company Z. Author D is the inventor of patent X.

If no conflicts exist, the authors should state:

Conflicts of Interest: The authors declare no conflicts of interest.

[Return to top]

Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether they are properly prepared and whether they follow the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals' *Editor-in-Chief* or *Associate Editors* to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board Members* and Guest Editors of the journal. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past five years and should not currently work or collaborate with any of the institutions of the co-authors of the submitted manuscript.

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The journal operates optional open peer-review: *Authors are given the option for all review reports and editorial decisions to be published alongside their manuscript. In addition, reviewers can sign their review, i.e., identify themselves in the published review reports.* Authors can alter their choice for open review at any time before publication, but once the paper has been published changes will only be made at the discretion of the *Publisher* and *Editor-in-Chief*. We encourage authors to take advantage of this opportunity as proof of the rigorous process employed in publishing their research. To guarantee impartial refereeing, the names of referees will be revealed only if the referees agree to do so, and after a paper has been accepted for publication.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.

- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. Usually, only one round of major revisions is allowed. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments.

- *Reject and Encourage Resubmission*:
  If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.

- *Reject*:
  The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments. The *Managing Editor* of the journal will forward the manuscript and related information (including the identities of the referees) to the Editor-in-Chief, Associate Editor, or Editorial Board member. The academic Editor being consulted will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage is final and cannot be reversed.

In the case of a special issue, the *Managing Editor* of the journal will forward the manuscript and related information (including the identities of the referees) to the *Editor-in-Chief* who will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be reversed.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the **www.mdpi.com** website.

[Return to top]

Promoting Equity, Diversity and Inclusiveness Within MDPI Journals

Our Managing Editors encourage the Editors-in-Chief and Associate Editors to appoint diverse expert Editorial Boards. This is also reflective in our multi-national and inclusive workplace. We are proud to create equal opportunities without regard to gender, ethnicity, sexual orientation, age, religion, or socio-economic status. There is no place for discrimination in our workplace and editors of MDPI journals are to uphold these principles in high regard.

[Return to top]

Resource Identification Initiative

To improve the reproducibility of scientific research, the **Resource Identification Initiative** aims to provide unique persistent identifiers for key biological resources, including antibodies, cell lines, model organisms and tools.

We encourage authors to include unique identifiers - RRIDs- provided by the **Resource Identification Portal** in the dedicated section of the manuscript.

To help authors quickly find the correct identifiers for their materials, there is a single **website** where all resource types can be found and a ‘cite this’ button next to each resource, that contains a proper citation text that should be included in the methods section of the manuscript.