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EXTRACTOS DE PLANTAS PARA EL CONTROL DE *STAPHYLOCCOCUS AUREUS* Y *SALMONELLA* SPP. RESISTENTES A ANTIBIOTICOS

Tesis para optar al grado de Magíster en Ciencias Agronómicas

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CHILLÁN-CHILE
2024

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RESUMEN

Existen enfermedades transmitidas por alimentos (ETAs), causadas por bacterias que pueden afectar tanto a animales como a humanos. Dentro de estas bacterias está *Staphylococcus aureus* y *Salmonella* spp., las cuales pueden adquirir resistencia antimicrobiana (RAM), dificultando su control y propagación en la cadena productiva de alimentos. El objetivo de esta investigación fue determinar el efecto antimicrobiano de extractos de plantas frente a *Staphylococcus aureus* y *Salmonella* spp. resistentes a antibióticos, aisladas en la cadena productiva avícola. Tras recolectar 356 muestras de gallinas y pollos de carne, carne de pollo y huevos, se confirmó la presencia de ambas bacterias mediante cultivo selectivo y PCR. Se determinó la diversidad genética de las cepas aisladas a través de ERIC-fingerprinting, seleccionando una cepa de cada cluster. Las cepas seleccionadas fueron sometidas a análisis de susceptibilidad a antibióticos y a extractos de plantas (aceite esencial de orégano, extracto de orujo de uva y extracto de cáscara de castaña) mediante el método de difusión en disco. Se encontró una prevalencia del 13.64% para *S. aureus* y del 6.53% para *Salmonella* spp. en la cadena productiva avícola, con la identificación de cepas multirresistentes a antibióticos en ambos casos. Se observó que el aceite esencial de orégano y el extracto de cáscara de castaña exhibieron una actividad antibacteriana contra ambas especies, mientras que el extracto de orujo de uva no demostró efecto inhibitorio. Por lo tanto, el aceite esencial de orégano y el extracto de cáscara de castaña podrían ser alternativas para controlar cepas resistentes a antibióticos en la cadena de productiva de carne de aves y huevos.

SUMMARY

There are foodborne diseases caused by bacteria that can affect both animals and humans. These bacteria include *Staphylococcus aureus* and *Salmonella* spp, which can acquire antimicrobial resistance (AMR), making their control and spread in the food production chain difficult. The aim of this study was to determine the antimicrobial activity of plant extracts against antibiotic-resistant *Staphylococcus aureus* and *Salmonella* spp. isolated in the poultry production chain. After collecting 356 samples of broilers, chicken meat and eggs, the presence of both bacteria was confirmed by selective culture and PCR. The genetic diversity of the isolated strains was determined by ERIC fingerprinting, with one strain selected from each cluster. The selected strains were subjected to susceptibility analysis to antibiotics and plant extracts (oregano essential oil, grape pomace extract and chestnut shell extract) using the disc diffusion method. A prevalence of 13.64% was found for *S. aureus* and 6.53% for *Salmonella* spp. in the poultry production chain, with the identification of multidrug resistant (MDR) strains in both cases. It was observed that oregano essential oil and chestnut shell extract showed antibacterial activity against both species, while grape pomace extract did not show any inhibitory effect. Therefore, oregano essential oil and chestnut shell extract could be alternatives to control antibiotic resistant strains in the poultry meat and egg production chain.

CAPÍTULO 1. INTRODUCION GENERAL

La resistencia antimicrobiana (RAM) es una amenaza sanitaria global, que, si no se toman las medidas adecuadas a tiempo, puede tener efectos negativos para la salud humana, animal y ambiental (Serra, 2017). Las bacterias patógenas han desarrollado resistencia a antibióticos como quinolonas, tetraciclinas, y aminoglucósidos, amenazando de esta forma los tratamientos farmacológicos (Liu *et al.,* 2021). Se estima que al año 2050, aproximadamente 10 millones de personas podrían morir cada año a causa de RAM, con un costo económico mundial de 86 billones de dólares. Según la Organización Mundial de la Salud (OMS), la RAM se produce porque las bacterias logran adaptarse a través de diferentes mecanismos y crecer en presencia del antibiótico (Mirabal, 2020). Está demostrado que diferentes cepas bacterianas poseen la capacidad de desarrollar resistencia, por motivos de mal uso de los antimicrobianos y de utilización de antibióticos a largo plazo (Galo *et al.,* 2020).

Durante los últimos años, no ha existido un avance importante en desarrollar nuevas alternativas a los antibióticos (Álvarez *et al.,* 2021). Sin embargo, se requieren más investigaciones para el desarrollo de estrategias que utilicen nuevos tratamientos para enfermedades infecciosas, incluyendo la utilización de productos naturales (Navarrete *et al.,* 2020).

En la actualidad, existe interés en la búsqueda de nuevos medicamentos con capacidad antimicrobiana (Bonilla *et al*., 2016). En este contexto, la OMS hace un llamado a que los países desarrollen políticas basadas en conocimiento y estrategias para la utilización de plantas medicinales (Marinho *et al.*, 2021). Existen estudios que indican que los extractos de plantas pueden ser una alternativa para la producción de nuevos antimicrobianos (Demirtaş *et al*., 2018; Tayel *et al.,* 2018; Rehman *et al*., 2016; *Gnat* et al., 2017), especialmente para aquellas bacterias que exhiben RAM (Tayel *et al*., 2018; Rehman *et al*., 2016), además poseen menor costo económico y un efecto eficiente, debido a que los compuestos activos tienen la capacidad de retrasar o inhibir la replicación bacteriana (Cruz *et al*., 2016). Las plantas son una gran fuente de fitoquímicos, en donde destacan los compuestos fenólicos. Los compuestos fenólicos son metabolitos secundarios que las plantas sintetizan principalmente bajo estrés (ambiental) o como mecanismo de defensa frente a determinados patógenos (Parola *et al.,* 2021). Las capacidades tanto antioxidantes como antimicrobianas son atribuidas a los compuestos fenólicos (Bano *et al*., 2021). La interacción de compuestos fitoquímicos contenidos en aceites esenciales, constituidos generalmente por compuestos aromáticos, y sustancias orgánicas con actividad antimicrobiana conforman un mecanismo de defensa que tienen las plantas contra enfermedades (Zhu *et at*., 2021). El aceite esencial de orégano (*Origanum vulgare* L.) posee actividad antimicrobiana y antioxidante, lo cual se atribuye a sustancias como el timol y el carvacrol (Dutra *et al*., 2019), que presentan actividad antimicrobiana frente a varios patógenos que son transmitidos mediante alimentos (An *et al.,* 2021). Algunos residuos o subproductos provenientes de la industria agrícola son una fuente valiosa de compuestos activos que pueden ser utilizados como antioxidantes y antimicrobianos (An *et al.,* 2021). Dentro de estos está el orujo de uva (*Vitis vinífera*), obtenido en la elaboración del vino, el cual es una buena fuente de compuestos bioactivos (ácidos fenólicos, flavonoles, flavanoles, antocianinas, taninos y estilbenos) responsables de diversas actividades biológicas, entre ellas la acción antimicrobiana (Goulas *et al*., 2021). Otro subproducto o residuo de la agroindustria corresponde a la cáscara de castaña (*Castanea sativa*), que posee una gran cantidad de compuestos bioactivos, destacando los compuestos fenólicos (Hu *et al*., 2021). Los compuestos fenólicos detectados en cáscara de castaña son ácidos fenólicos (elágicos y galicos), flavonoides (rutina, quercetina y apigenina) y taninos (Lameirão *et al.,* 2020). El extracto de la cáscara de castaña también posee efecto antimicrobiano frente a diversos patógenos que se transmiten mediante alimentos (Park *et al*., 2016). Por lo tanto, los extractos de plantas con actividad antimicrobiana podrían utilizarse en el control de bacterias patógenas, de importancia en salud humana y animal.

Existe una asociación entre la producción de carne de aves de corral y la salud humana, lo que está generando problemas en la industria avícola en materia de seguridad de los alimentos, en relación con la mitigación de la RAM (Mwandinge *et al*., 2021). El consumo de alimentos contaminados con patógenos (bacterias, virus y parásitos) o sustancias químicas dañinas puede causar brotes de enfermedades trasmitidas por alimentos (ETAs) (Qosimah *et al*., 2021). Entre las bacterias más comunes que producen ETAs se encuentran *Salmonella*, *Escherichia coli* enterohemorrágica, *Campylobacter*, *Shigella*, *Listeria* y *Vibrio cholerae* (WHO, 2020), siendo *Salmonella* y *Escherichia coli* algunos microorganismos que comúnmente presentan RAM (Andrew *et al*., 2020). Bacterias asociadas a la producción avícola como *Salmonella* y *Campylobacter* spp. son frecuentemente notificados como causantes de ETAs (Hafez & Attia, 2020).

Algunas cepas de la bacteria *Staphylococcus aureus*, producen enterotoxinas que pueden provocar brotes de intoxicación alimentaria en humanos. De esta forma, *S. aureus* enterotoxigénico resistente a antibióticos presente en alimentos es una amenaza para la salud humana (Ma *et al*., 2018).

El uso de antibióticos durante los últimos años en la cadena productiva avícola permitía garantizar rentabilidad en las granjas, eficiencia y altos niveles de producción, además del bienestar de las aves. Esta práctica se ha asociado a la emergencia y propagación de cepas bacterianas resistentes a antibióticos, la aparición de estas mismas cepas bacterianas en los productos finales, desequilibrios en la flora normal del intestino del animal y la preocupación científica. Debido a estos motivos, durante enero del año 2006, la Unión Europea prohibió el uso de antibióticos como promotores de crecimiento (APC) en la producción animal (Maya *et al*., 2021). En tanto a la situación nacional, la prohibición de APC ocurre durante el año 2007 (Res. SAG No. 1992, 2006).

Existen antecedentes que demuestran la capacidad antimicrobiana de algunos extractos naturales frente a ciertas bacterias, pero no han sido evaluados en diferentes etapas de la cadena productiva de alimentos. Por este motivo, se requiere determinar la susceptibilidad de bacterias patógenas resistentes a antibióticos a extractos de plantas, para poder contar con alternativas para contener la RAM y mejorar la inocuidad alimentaria. Durante los últimos años *S. aureus* enterotoxigénico y *Salmonella* spp. se han notificado de forma reiterada en la cadena productiva de productos avícolas. Estas bacterias comprometen la salud humana, inocuidad y calidad de los alimentos, por lo tanto, se requieren más estudios para poder controlar de manera efectiva la disipación de ciertas bacterias con resistencia a los antimicrobianos en el ecosistema.

HIPÓTESIS

Extractos de plantas tienen efecto antimicrobiano contra *S. aureus* y *Salmonella* spp. resistentes a antibióticos, aisladas en la cadena productiva avícola.

OBJETIVO GENERAL

Evaluar el efecto antimicrobiano de extractos de plantas frente a *S. aureus* y *Salmonella* spp. resistentes a antibióticos, aisladas en la cadena productiva avícola.

OBJETIVOS ESPECIFICOS

- Determinar la prevalencia de *S. aureus* y *Salmonella* spp. resistentes a antibióticos, aisladas en la cadena productiva avícola.
- Determinar la actividad antimicrobiana de extractos vegetales de interés agronómico y agroindustrial.

REFERENCIAS

Álvarez, F.J., Rodríguez, J.C., Borrás, F., Barrajón, E., Micol, V. 2021. The antimicrobial capacity of *Cistus salviifolius* and *Punica granatum* plant extracts against clinical pathogens is related to their polyphenolic composition. Scientific Reports. 11 (1): 588.

An, J., Wang, L., Lv, M., Wang, J., Cai, Z., Wang, Y., Zhang, S., Yang, Q., Fu, Y. 2021. An efficiency strategy for extraction and recovery of ellagic acid from waste chestnut shell and its biological activity evaluation. Microchemical Journal. 160: 105616.

Andrew, L., Mohammed, Z., Manyelo, T., Mabelebele, M. 2020. The Current Status of the Alternative Use to Antibiotics in Poultry Production: An African Perspective. Antibiotics (Basel). 9 (9): 594.

Bano, A., Gupta, A., Rai, S., Sharma, S. 2021. Elucidation of Bioactive Potential of Two Commonly Grown North Indian *Psidium guajava* viz., Lalit and Shweta against Pathogenic Foodborne and MDR Bacteria. Biointerface research in applied chemistry .11 (6): 14090- 14102.

Bonilla, D., Mendoza, Y., Moncada, C., Murcia, O., Rojas, Á., Calle, J., Pinzón, R., Nerio, L. 2016. Efecto del aceite esencial de *Rosmarinus officinalis* sobre *Porphyromonas gingivalis* cultivada in vitro. Revista Colombiana de Ciencias Químico – Farmacéuticas. 45 (2): 275-287.

Cruz, A., Brito, I., Sobral, M., Sousa, A., Alves, E., Andreza, R., Ferreira, S.,Costa, R., Figueredo, F., Tam, T., Macêdo, R., Ribeiro, T., Leandro, L., Oliveira, C., Tintino, S., Costa, M., Aquino, P. 2016. Avaliação da atividade antibacteriana e moduladora dos extratos metanólico e hexânico da folha de *Allium cepa.* Revista Ciencias de la Salud. 14 (2): 191-200.

Demirtaş, A., Öztürk, H., Pişkin, I. 2018. Overview of plant extracts and plant secondary metabolites as alternatives to antibiotics for modification of ruminal fermentation. Ankara Universitesi Veteriner Fakultesi Dergisi. 65: 213-217.

Dutra, T.V., Castro, J.C., Menezes, J., Ramos, T.R., Prado, I.N., Junior, M.M., Mikcha, J.G., Filho, B.A. 2019. Bioactivity of oregano (*Origanum vulgare*) essential oil against *Alicyclobacillus* spp. Industrial Crops and Products. 129: 345-349.

Galo, I. D. C., Lima, B. E. De, Santos, T. G., Braoios, A., Prado, R. P., Santos, W. G. Dos. 2021. *Staphylococcus aureus* growth delay after exposure to low fluencies of blue light (470 nm). Brazilian Journal of Biology. 81 (2): 370-376.

Gnat, S., Majer-Dziedzic, B., Nowakiewicz, A., Trościańczyk, A., Ziółkowska, G., Jesionek, W., Choma, I., Dziedzic, R., Zięba, P. Antimicrobial activity of some plant extracts against bacterial pathogens isolated from faeces of red deer (*Cervus elaphus*). Polish Journal of Veterinary Sciences. 20 (4): 697-706

Goulas, V., Stavrou, K., Michael, C., Botsaris, G., Barbouti, A. 2021. The Potential of Sun-Dried Grape Pomace as a Multi-Functional Ingredient for Herbal Infusion: Effects of Brewing Parameters on Composition and Bioactivity. Antioxidants. 10 (4): 586.

Hafez, H. M., Attia, Y. A. 2020. Challenges to the Poultry Industry: Current Perspectives and Strategic Future After the COVID-19 Outbreak. Frontiers in veterinary science. 7: 516

Hu, M., Yang, X., Chang, X. 2021. Bioactive phenolic components and potential health effects of chestnut shell: A review. Journal of Food Biochemistry. 45: e13696.

Lameirão, F., Pinto, D., Vieira, E., Peixoto, A., Freire, C., Sut, S., Dall'Acqua, S., Costa, P., Delerue-Matos, C., Rodrigues, F. 2020. Green-Sustainable Recovery of Phenolic and Antioxidant Compounds from Industrial Chestnut Shells Using Ultrasound-Assisted Extraction: Optimization and Evaluation of Biological Activities In Vitro. Antioxidants (Basel). 9 (3): 267

Liu, B., Zhang, X., Ding, X., Wang, Y., Zhu, G. 2021. Regulatory mechanisms of sub-inhibitory levels antibiotics agent in bacterial virulence. Applied Microbiology and Biotechnology.105 (9): 3495-3505.

Ma, Y., Zhao, Y., Tang, J., Tang, C., Chen, J., Liu, J. 2018. Antimicrobial susceptibility and presence of resistance & enterotoxins/enterotoxin-likes genes in*Staphylococcus aureus* from food. CyTA - Journal of Food. 16(1): 76-84.

Marinho, T. A., Oliveira, M. G., Menezes-Filho, A. C. P., Castro, C. F. S., Oliveira, I. M. M., Borges, L. L., Melo-Reis, P. R., & Silva-Jr, N. J. 2021. Phytochemical characterization, and antioxidant and antibacterial activities of the hydroethanolic extract of *Anadenanthera peregrina* stem bark. Brazilian Journal of Biology. 82: e234476 [En línea].

Maya, C., Madrid, T., Parra, J. 2021. Efecto de *Bacillus subtilis* sobre metabolitos sanguíneos y parámetros productivos en pollo de engorde. Biotecnología en el Sector Agropecuario y Agroindustrial. 19 (1): 105-116.

Ministerio de agricultura. 2006. Establece nómina de aditivos autorizados para la elaboración y fabricación de alimentos y suplementos para animales y deroga resoluciones que indica. NCh 1992: of. 2006. Santiago, Chile

Mirabal, G.A. 2020. Resistencia a los antimicrobianos en hospitales infantiles: perspectiva y realidad. Revista Biomedica. 31 (1): 1-2.

Navarrete N., Pita, E., Sánchez, R., Giraldo S., Bernal M. 2020. Actividad in vitro de los extractos etanólicos de *Lantana camara* L., *Petiveria alliacea* L. y *Lippia dulcis* T. frente a bacterias patógenas. Nova. 18 (33): 53-71.

Organización mundial de la salud (OMS). 2020. Inocuidad de alimentos [en línea]. < https://www.who.int/es/news-room/fact-sheets/detail/food-safety > [Consulta: 17 mayo 2021].

Park, S.M., Kang, J.H., Son, H.J., Oh, D.H., Min, S.C., Song, K.B. 2016. Combined treatments of chestnut shell extract, fumaric acid, and mild heat to inactivate foodborne pathogens inoculated on beetroot (*Beta vulgaris* L.) leaves. Food Sience and Biotechnology. 25 (4): 1217-1220.

Parola I., Guevara, R., Feregrino, A., Reynoso, R., Pérez, I., Ocampo, R., Rojas, A., Luna, F., Tovar, E. 2021. Phenolic compounds and antioxidant activity of methanolic extracts from leaves and flowers of chilcuague (*Heliopsis Longipes* Asteraceae). Botanical Sciences. 99 (1): 149-160.

Qosimah, D., Rosyidi, D., Radiati, L.E., Amri, I.A., Prasetyo, D., Permata, F.S., Anisa, A.K. 2021. Green Coffea robusta (*Coffea canephora*) from Lampung province effect toward free radicals in chickens infected with Salmonella enteritidis bacteria. Open Veterinary Journal. 11 (1): 61-69.

Rehman, S., Mujtaba Ghauri, S., Sabri, A. N. 2016. Impact of Plant Extracts and Antibiotics on Biofilm Formation of Clinical Isolates From Otitis Media. Jundishapur journal of microbiology. 9 (1): e29483.

Serra, M. (2017). La resistencia microbiana en el contexto actual y la importancia del conocimiento y aplicación en la política antimicrobiana. Revista Habanera de Ciencias Médicas. 16 (3): 402-419.

Tayel, A., El-Sedfy, M., Ibrahim, A., Moussa, S. 2018. Application of Quercus infectoria extract as a natural antimicrobial agent for chicken egg decontamination. Revista argentina de microbiología. 50 (4): 391-397.

Zhu Y., Li, C., Cui, H., Lin, L. 2021. Encapsulation strategies to enhance the antibacterial properties of essential oils in food system. Food Control. 123 (2): 107856

Capitulo 2. Plant extracts for the control of antibiotic-resistant *Staphylococcus aureus* isolated from poultry

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Abbreviated running headline: Plant extracts against *S. aureus* from poultry

Revista: Journal of Applied Microbiology

Fecha de envío:29/03/24

Abstract

Aim: To determine the susceptibility of *Staphylococcus aureus* strains of poultry origin to antibiotics, oregano essential oil (EO) (*Origanum vulgare* L.), chestnut shell extract (*Castanea Sativa*) and grape pomace extract (*Vitis vinifera*).

Methods and Results: Samples were taken from hens and broiler chickens (n=120), poultry meat (n=98) and eggs (n=138). *S. aureus* was isolated by selective culture method and confirmed by PCR (*nuc* gen). Eight *S. aureus* strains were selected using ERIC-fingerprinting PCR. Selected strains were subjected to antimicrobial susceptibility testing using the disk diffusion method. The total prevalence of *S. aureus* was 13.64%. A higher prevalence (5.34%) was found in samples from conventional system compared with cage-free system and in non-packaged meat compared with packaged meat ($P \leq 0.05$). Four strains were multidrug resistant (MDR). Oregano EO and chestnut shell extract had antibacterial activity. However, grape pomace extract did not exhibit anti-staphylococcal activity.

Conclusions: Oregano EO and chestnut shell extract have inhibitory activity against *S. aureus* strains of poultry origin, and grape pomace extract has not inhibitory effect.

Impact statement: This study provides information on the prevalence of *S. aureus* in poultry and on the potential use of plant extracts to control antibiotic-resistant *S. aureus* strains in the food chain.

Keywords: antimicrobial resistance (AMR), *Staphylococcus aureus*, poultry, oregano essential oil (EO), chestnut shell, grape pomace.

Introduction

Antimicrobial resistance (AMR) is a global health threat, which, if appropriate measures are not taken in time, can have negative effects on human, animal and environmental health (Serra, 2017). AMR has increased exponentially during the last few years, due to the excessive and indiscriminate use of antibiotics (Camou *et al.*, 2017). Pathogenic bacteria have developed resistance to antibiotics such as quinolones, tetracyclines, and aminoglycosides, thus threatening pharmacological treatments (Liu *et al*., 2021). It is estimated that by 2050, approximately 10 million people could die each year from AMR, with a global economic cost of US$86 trillion (Mirabal, 2020). During the last few years, there has been no significant progress in developing new alternatives to antibiotics (Álvarez *et al*., 2021), and more research is required to develop new strategies to control and mitigate AMR, including the use of natural products (Navarrete *et al*., 2020). In this context, WHO calls on countries to develop knowledge-based policies and strategies for the use of medicinal plants (Marinho *et al* 2021).

Staphylococcus aureus is a bacterium that has developed the ability to generate several antibiotic resistance mechanisms to antibiotics (Guillén *et al*., 2016). Moreover, it is widely distributed in nature, and it is found in diverse environments. Although considered part of the normal bacterial flora on human and animal skin and mucous membranes, this bacterium is an opportunistic pathogen capable of causing a wide range of infections in humans and animals, including skin infections, pneumonia, septicemia and foodborne diseases (Zendejas *et al*., 2014). This bacterium is highly resistant to many adverse conditions, despite not forming spores

(Guillén *et al*., 2016). In addition, the presence of *S. aureus* in the poultry meat and egg industry, especially in raw meat or poultry products, is one of the most common causes of food poisoning worldwide (Pyzik *et al*., 2014). There are studies that indicate that some plant extracts can be an alternative to develop new antimicrobials (Demirtaş *et al*., 2018; Tayel *et al*., 2018; Rehman *et al*., 2016; Gnat *et al*., 2017), especially for those bacteria that exhibit AMR (Tayel *et al*., 2018; Rehman *et al*., 2016). In addition, they are cheaper and show an efficient effect, because the bioactive compounds have the ability to delay or inhibit bacterial replication (Cruz *et al*., 2016). Plants are a great source of phytochemicals, such as phenolic compounds, which are secondary metabolites synthesized by plants mainly under environmental stress or as a defense mechanism against certain pathogens (Parola *et al*., 2021). Both antioxidant and antimicrobial capacities are attributed to phenolic compounds (Bano *et al.,* 2021). The interaction of these phytochemical compounds can effectively inhibit the growth of pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* (Parola *et al.,* 2021). These studies are essential to effectively control the spread of resistant bacteria in the ecosystem and ensure the safety of poultry products (Álvarez et al., 2021). Therefore, the aim of this study was to determine the prevalence and the susceptibility of Staphylococcus aureus strains of poultry origin to antibiotics, essential oil (EO) of oregano, chetsnut shell extract and grape pomace extract.

Materials and methods

Sampling

A total of 356 samples were collected from different stages of the poultry meat and egg production chain between December 2021 and January 2022, in region of Ñuble and region of Biobío, Chile. Sample size (Suppl. data S1) was determined using equation 1 (Moore, 2007).

$$n \geq \left(\frac{z}{m}\right)^2 \times \hat{p}\left(1 - \hat{p}\right) \qquad (1)$$

Where n is the sample size, z is the standard score in the normal distribution frequency, m corresponds to the margin of error, and $\hat{p}$ is the prevalence of pathogens presenting AMR obtained in other studies. The confidence level of the interval corresponds to 95%, with z=1.96 and m=0.05 (considering an interval no greater than 10%).

Samples were collected from laying hens and broiler chicken (farms) (skin) (n=120), whole and cut-up chicken (supermarkets and butcher shops) (meat) (n=98), eggs (farms, supermarkets and retail stores) (n=138). The legal and ethical approval was obtained from the Ethics, Bioethics and Biosecurity Committee of the University of Concepcion, Chile, prior to conducting sampling (Suppl. data S2). Briefly, the animals were immobilized inside cages and skin samples were obtained by passing a sterile swab under one wing. For egg samples, the entire eggshell was sampled with a sterile swab. Whole and cut-up chicken meat samples were randomly selected and purchased. Samples were immediately transported at 4°C and processed.

***S. aureus* isolation**

The isolation of *S. aureus* was carried out through selective enrichment and culture techniques. Briefly, swab samples were suspended in 10 mL of sterile buffered peptone water (BPW), and meat samples (25 g) with MHB+6.5% NaCl (225 mL) were placed in a sterile filter bag and homogenized using a lab blender (BagMixer400 P; Interscience, St. Nom, France) at 8 strokes/s for 90 s. Following incubation (24 h at 35±2°C) the suspension were streaked onto Baird Parker agar (BPA) supplemented with tellurite egg yolk and incubated for 48 h at 35±2°C. Presumptive positive colonies (black colonies with a clear halo) were selected and streaked onto tryptic soy agar (TSA), followed by incubation for 24 h at 35±2°C. Presumptive *S. aureus* colonies were stored at -80°C in cryotubes containing brain heart infusion broth (BHI) with 20% glycerol.

DNA Extraction

DNA from presumptive *S. aureus* strains was extracted using the boiling method proposed by Ngamwongsatit *et al.* (2008). Briefly, each strain was streaked on TSA and incubated for 24 h at 35±2°C. Then, 1 to 3 colonies were inoculated into 1 mL of trypticase soy broth (TSB) and centrifuged at 5000 x *g* for 2 min (Prism R™ Refrigerated MicroCentrifuge; Labnet; USA). The pellet was resuspended in 500 µL of DNA/RNAase-free H_2O and centrifuged at 5000 x *g* for 2 min. Subsequently, the pellet was resuspended in 100 µL of DNA/RNAase-free H_2O and kept at 100°C for 10 min (AccuBlock™ digital dry baths; Labnet; USA) followed by centrifugation at 1000 x *g* for 5 min. The resulting supernatant was stored at -20°C. DNA concentration was adjusted at A260/A280 = 1.8 – 2.0 (10 – 100 ng /mL).

Identification/confirmation of *S. aureus*

The *nuc* gene (*S. aureus* specific region of thermonuclease, 279 bp) was identified by PCR to confirm *S. aureus* strains. The primers were: *nuc*1F (5' GCGATTGATGGTGATACGGTT 3') and *nuc*2R (5' AGCCAAGCCTTGACGAACTAAAGC 3'). PCR reactions were carried out in a volume of 25 µL, including: 2.5 µL of DNA template (<250 ng), 12.5 µL 1X GoTaq Master Mix (200 µM dNTPs, 3 mM $MgCl_2$) (Pro-mega, Madison, WI, USA) (Pro-mega), 1 µL of each primer (0,4 µM) (Invitrogen Integrated DNA Technologies, Inc., Coralville, IA, USA). PCR conditions were adjusted following the protocol described by Enright *et al.* (2000) using an thermocycler (Labnet™ Thermocycler MultiGene™ OptiMax TC9610-230, Hamburg, Germany). Subsequently, 10 µL of the PCR product was loaded on 1.5% agarose gels in 1X TAE with 10 µL SafeView as DNA-intercalating dye. Two positive controls *S. aureus* ATCC 25923 and Methicillin-Resistant *S. aureus* (MRSA) ATCC 43300 and two negative control *Salmonella* ATCC 13076 and DNA/RNAase-free H_2O were included. A molecular weight marker 100-bp ladder (Maestrogen, Inc., Las Vegas, NV, USA) was included in each gel. Electrophoresis was carried out at 100 V in TAE 1X for 1 h. Bands were visualized using a UV transilluminator at 312 nm (BIOTOP TU1002; Shangai Bio-Tech Co. Ltd. Shangai, China).

ERIC-Fingerprinting PCR for *S. aureus*

The genetic diversity of *S. aureus* isolates was assessed by ERIC-PCR molecular fingerprinting using the primers Eric2F (5' AAGTAAGTGACTGGGGTGAGCG 3') and Eric1R (5' ATGTAAGCTCCTGGGGATTCAC 3') (Versalovic *et al*., 1991). Reference strains of *S. aureus* ATCC 25923 and ATCC 43300 were used as positive controls and *Salmonella enterica* subsp. Enterica ATCC 13076 was used as negative control.

The ERIC PCR mix was performed using 2.0 µL of 50 ng DNA template, 1.25 µL of forward and reverse primers 10 µM, 12.5 µL of GoTaq® Colorless Master Mix (Promega) and 9.5 µL of DNA/RNAase-free H_2O (Promega) to a total of 25 µL. PCR conditions were adjusted according to Akindolire *et al*. (2018).

The PCR products were subjected to electrophoresis in 2% (w/v) agarose gels with a previous standardization of the DNA concentration for each sample. Each lane was charged with 15 µL of PCR product and 3 µL of loading dye supplemented with SafeView Plus DNA gel stain 20,000X (Fermelo Biotec). The marker used was 100-bp Plus DNA ladder (Maestrogen). Electrophoresis was carried out at 100V for 3 h with 1xTAE (40mM Tris-Acetate, 1mM EDTA, pH 8.0). Bands were visualized in a UV transilluminator 320 nm.

The banding patterns were manually analyzed to scan for the presence (scores 1 or 0) of PCR products of specific molecular sizes using the Gerding *et al*. (2013) protocol. The matrix was then analyzed with AFPL SURV version 1.0 (Vekemans *et al*., 2002) to calculate the genetic distance among isolates. The distance matrix was subjected to UPGMA cluster analyses using the NEIGHBOR application from the PHYLIP software package. The cladograms were visualized in MEGA7 (Kumar *et al.*, 2016) and strains were selected according to their genetic diversity.

Susceptibility to antibiotics

Selected *S. aureus* strains by ERIC-fingerprinting were subjected to antibiotic susceptibility testing using the disk diffusion method. Briefly, 0.1 mL of a bacterial solution (0.85% saline NaCl solution, McFarland 0.5 ≈ 10^8 CFU) was streaked uniformly on Mueller-Hinton agar (MHA) supplemented with 2% NaCl (MHA+2% NaCl) using a sterile cotton swab. After placing the disks on the agar surface, plates were incubated at 35 ± 2 °C for 24 h. For the interpretation of the results, the criteria of the Clinical and Laboratory Standard Institute (CLSI, 2020) was used. For susceptibility to cefoxitin, disks containing 30 µg were used (CLSI 2020).

Twelve antibiotics from ten different classes were examined: oxacillin, cefoxitin, penicillin, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, ceftaroline, oxytetracycline, vancomycin, chloramphenicol, ciprofloxacin and gentamicin.

Characterization of plant extracts

The following extracts were evaluated: oregano essential oil (EO) (chemotype carvacrol, 99.9% of carvacrol; R.C. Treatt & Co Ltd, Suffolk, UK), grape pomace extract (Carmenere, Merlot and Syrah) and chestnut shell extract. Quantification of total phenolic compounds content of grape pomace and chestnut shell extracts was carried out following the method of Singleton *et al.* (1999), a modification of the Folin-Ciocalteu method. In this procedure, the Folin-Ciocalteu reagent and 20% (m/v) sodium carbonate solution were used. The absorbance of the samples was measured at 765 nm using a microplate multilector. The results obtained were expressed as Gallic Acid Equivalents per gram of extract (GAE/g extract).

Susceptibility to plant extracts

Oregano EO was prepared in absolute ethanol (99.8%; Merck, Darmstadt, Germany) to obtain a concentration of 5%. Chestnut shell extract was prepared with absolute ethanol to reach a concentration of 40%. Grape pomace extract was tested in ethanol:distilled water at 60:40 v/v.

Plant extracts were sterilized using a 0.22 µm filter (EDLAB CA Syringe Filter). Then, the antimicrobial activity of these agents was evaluated by the disk diffusion method using sterile paper disks (Whatman No. 1, 6 mm diameter) according to the procedure described by Rota *et al.* (2008).

Bacterial suspensions were prepared in trypticase soy broth (TSB) with an optical density of 0.1 (OD_{600} = 0.1) measured in a UV/VIS spectrophotometer (BioTek Epoch Microplate Spectrophotometer, Epoch Life Science, Inc., USA). Then, a volume of 0.1 mL of this suspension was inoculated onto TSA plates.

Sterile disks impregnated with 15 µL of each plant extract solution were placed on the inoculated plates, followed by an incubation at 35±2 °C for 24 h. The inhibition criteria (including disk diameter) were as follows: an inhibition zone ≥20 mm indicated strong inhibition; between <20 and 12 mm, moderate/slight inhibition; and <12 mm indicated lack of inhibition (Rota *et al.* 2008). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the dilution method according to Rota *et al.* (2004).

Isolates were inoculated on TSA agar plates and incubated at approximately 35±2 °C for 24 h. The colonies were then transferred to trypticase soy broth (TSB) in 2 mL Eppendorf tubes (OD_{600}=0.1). An aliquot of this solution was inoculated into serial dilutions of each plant extract in triplicate. Ethanol and distilled water were used as controls to validate the results. After inoculation, plates were incubated for 24 h at 35±2 °C. After incubation, the OD was read and samples with an OD equal to or higher than the initial OD were selected and inoculated on TSA agar plates and incubated for 24 h at 35±2 °C to allow growth in triplicate.

Statistical analysis

The Chi-square method or Fisher's exact test was used to determine the differences in the prevalence of *S. aureus* between different type of samples taken from the poultry production chain ($P \leq 0.05$) (Moore, 2007). The sizes of inhibition zones were assessed using descriptive statistics. These criteria were based on the approach by Rota *et al.* (2008).

Results

Table 1 shows the prevalence of *Staphyloccocus aureus* in the poultry production chain. The prevalence of *Staphylococcus aureus* in poultry was 13.64% (48/356). These positive samples were distributed among 14 isolates from animals, 15 from raw meat, and 19 from eggs. No statistically significant differences ($P > 0.05$) were found between the types of samples. Comparative analyses revealed significant differences within sample subgroups ($P \leq 0.05$).

Eight *S. aureus* strains were selected according to the genetic diversity determined by the ERIC fingerprinting (Suppl. data S3).

Table 2 shows the resistance profile of *S. aureus* strains isolated from the poultry production chain to antibiotics. Six strains exhibited resistance, one was obtained from skin samples, three from eggs, and two from meat.

The compound with the highest concentration of phenolic compounds was, Carvacrol in oregano EO (99.9%). As for grape pomace, Cabernet Sauvignon extract had the highest content of phenolic compounds (316.97 ± 22.57 mg GAE/g), followed by Carmenere (280.31 ± 8.84 mg GAE/g), and Merlot (259.75 ± 5.74 mg GAE/g). Grape pomace extracts of cabernet and merlot grape varieties did not show any biological activity against *S. aureus* strains. A significant higher concentration of phenolic compounds was obtained with chestnut shell extract, with 181.93 ± 13.10 mg GAE/g.

The chestnut shell extract showed a moderate inhibition activity against *S. aureus* strains (Table 2). Oregano EO, presented inhibitory activity against all *S. aureus* strains, with halos ≥20 mm, classified as strongly inhibitory activity. The MIC of oregano EO ranged from 0.0015 to 0.003% and the CMB ranged from 0.003 to 0.00625%. The MIC of chestnut shell extract ranged from 18.75 to 75 mg/mL and the CMB ranged from 37.5 to 150 mg/mL (Table 3).

Discussion

The findings in this study highlight the presence of *S. aureus* throughout the poultry chain, raising significant concerns regarding food safety and public health. This study identified significant differences in the prevalence of *S. aureus* within specific subgroups of the poultry and egg production chain, with a higher prevalence in conventional than cage-free system, and a higher prevalence in non-packaged than packaged meat (Table 1). Thus, identifying the distribution and potential risk factors associated with *S. aureus* contamination within the poultry production chain could allow the application of more effective control measures. In addition, a high prevalence of *S. aureus* in chicken meat has been observed in various geographical regions, highlighting the global significance of this issue. For instance, a study conducted in Egyptian markets revealed that 91.3% of chicken meat was contaminated with *S. aureus*, with an alarming 81.8% of isolates exhibiting multidrug resistance (MDR) (Eldin *et al*., 2023). In addition, in the markets of Ulaanbaatar, Mongolia, 35% of raw chicken meat samples were contaminated with *S. aureus* (Dorjgochoo *et al*., 2023). Similarly, studies in China have identified the presence of *S. aureus* in ready-to-eat foods, with a positivity rate of 12.5% (Yang *et al*., 2016). Therefore, the prevalence of *S. aureus* is a common problem in various food chains, underscoring the pressing need for improved food safety practices to control the spread of this pathogenic bacterium.

In this study, MDR strains (resistance to three or more classes of antibiotics) were identified in animals, eggs and meat (Table 2). In a study, conducted in Yunnan, China, which examined samples of two types of traditional Chinese cheese, Rubing and Rushan, 34.78% (8 of 23) of the isolates exhibited MDR (Adhita Sri Prabakusuma *et al.,* 2021). These results underscore the urgent need to implement more rigorous production practices and promote responsible antibiotic use.

It has been evidenced that phenolic compounds have the ability to act against pathogenic bacteria (Franco *et al*., 2022). Essential oils, due to their variability in chemical composition influenced by environmental and production factors, exhibit remarkable antibacterial properties attributed to compounds such as carvacrol and thymol (Turchi *et al.,* 2018). Oregano EO contains high levels of carvacrol and thymol, accounting for 78-85% of its fundamental chemical composition, giving it significant antioxidant and antimicrobial properties (Bagher and Mousavi, 2017), which was also observed in this study with the high inhibition of *S. aureus* strains (Table 2).

Chestnut shells are a by-product of the chestnut processing and have been found to harbor various molecules with beneficial properties, such as phenols, flavonoids, triterpenes, sugars, tannins, and brown pigments (Shao *et al.,* 2023), which could be responsible of the inhibitory activity of chestnut shell extract observed in this study (Table 2). These molecules possess antioxidant, antibacterial and anti-inflammatory capabilities, which underline their relevance as a valuable source of bioactive compounds. Grape pomace, obtained from the wine production, is composed of the skins and seeds. The classification of phenolic compounds present in grape pomace includes phenolic acids and flavonoids. However, several authors have also reported that grape pomace extracts do not show biological activity against Gram-positive bacteria (Table 2). For example, Alves *et al.* (2013) found no antimicrobial effect of grape skin extracts against certain pathogenic and food-borne spoilage bacteria, including *S. aureus* and *B. cereus*. Similar results were presented by Luchian *et al*. (2019) when evaluating the antibacterial activity of pomace extract from seven different grape varieties, observing a high antioxidant capacity but a negligible inhibitory effect on *S. aureus*. It is crucial to note that the bioactive properties of grape pomace extracts depend on their polyphenolic composition, which can vary according to the type of grape used, its geographical origin and the wine production method (Gerardi *et al*., 2021).

Bobrowski *et al*. (2023) reported the biological activity of chestnut shell extracts against Gram-positive bacteria, including *Bacillus cereus*, *Listeria monocytogenes* and *S. aureus*, with MIC 0.3 mg/mL and MBC >10 mg/mL, lower than the MIC and MBC obtained in this study (Table 3). In addition, Lee *et al*. (2022) evaluated chestnut inner shell extract effect against *S. aureus* using different solvents for extraction, water and 70% ethanol, obtaining MIC and MBC of 8 mg/mL for water and MIC of 8 mg/mL for 70% ethanol. These findings on the antimicrobial properties of chestnut shells offer a promising natural alternative to mitigate the presence of pathogenic bacteria such as *S. aureus* in poultry products. The application of these bioactive compounds could be crucial to strengthen food safety measures and decrease the risks associated with the presence of dangerous microorganisms.

In this study, the MIC of oregano EO ranged from 0.0015% to 0.003% (Table 3). This differs with previous studies, which reported different MIC and MBC values (Hao *et al*., 2021; Carhuallanqui *et al.,* 2020). Lofa *et al*. (2019), determined MIC values ranging from 0.01% to 0.02% and MBC values from 0.04% to 0.08% in antibiotic-resistant strains of *S. aureus* of swine origin. Therefore, the efficacy of oregano EO against antibiotic-resistant strains can vary and depends on different factors.

The main mechanisms of action of plant polyphenols is their ability to modify bacterial cell membrane permeability, affecting various metabolic and growth processes (Wang *et al*., 2023).

Conclusions

S. aureus and antibiotic-resistant strains are present in the poultry production chain, indicating that there are sources of contamination at different stages.

Plant extracts have antimicrobial effect against antibiotic-resistant *S. aureus* isolated from the poultry production chain, such as oregano EO with a high inhibitory activity, and chestnut shell extract with a moderately inhibitory activity on some strains.

Funding

This work was supported by the Research Project VRID No. 2021000173INV.

Conflict of Interest

No conflict of interest declared.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

Adhita Sri Prabakusuma, Zhu, J., Shi, Y., Ma, Q., Zhao, Q., Yang, Z., Xu, Y., & Huang, A. (2021). Prevalence and antimicrobial resistance profiling of *Staphylococcus aureus* isolated from traditional cheese in Yunnan, China. 3 Biotech. 12(1). doi: 10.1007/s13205-021-03072-4.

Akindolire, M.A., Kumar, A., Ateba, C.N., 2018. Genetic characterization of antibiotic-*resistant Staphylococcus aureus* from milk in the North-West

Province, South Africa. Saudi J. Biol. Sci. 25, 1348–1355. doi:10.1016/j.sjbs.2015.10.011.

Álvarez, F.J., Rodríguez, J.C., Borrás, F., Barrajón, E., Micol, V. 2021. The antimicrobial capacity of *Cistus salviifolius* and *Punica granatum* plant extracts against clinical pathogens is related to their polyphenolic composition. Scientific Reports. 11 (1): 588. doi: 10.1016/j.phymed.2021.153626.

Alves, D., Ana Augusta Salvador, Artur Smânia, Elza, Maraschin, M., & Sandra R.S. Ferreira. (2013). Antimicrobial activity and composition profile of grape (*Vitis vinifera*) pomace extracts obtained by supercritical fluids. Journal of Biotechnology. 164(3): 423–432. doi: 10.1016/j.jbiotec.2012.09.014.

Bagher, M., & Amin Mousavi Khanegha. (2017). Characterization of novel basil-seed gum active edible films and coatings containing oregano essential oil. Progress in Organic Coatings, 110: 35–41. doi: 10.1016/J.PORGCOAT.2017.04.041.

Bano, A., Gupta, A., Rai, S., Sharma, S. 2021. Elucidation of Bioactive Potential of Two Commonly Grown North Indian *Psidium guajava viz*., Lalit and Shweta against Pathogenic Foodborne and MDR Bacteria. Biointerface research in applied chemistry .11 (6): 14090- 14102. doi: 10.33263/BRIAC116.1409014102.

Bobrowski Rodrigues, Lavínia Veríssimo, Finimundy, T. C., Joana, Oliveira, I., José Alberto Gonçalves, Fernandes, I. P., Barros, L., Heleno, S. A., & Calhelha, R. C. (2023). Chemical and Bioactive Screening of Green Polyphenol-Rich Extracts from Chestnut By- Products: An Approach to Guide the Sustainable Production of High-Added Value Ingredients. Foods. 12(13).

2596–2596. doi: 10.3390/foods12132596.

Camou, T., Zunino, P., Hortal, M. 2017. Alarma por la resistencia a antimicrobianos: situación actual y desafíos. Revista Médica del Uruguay. 33(4): 104-127. doi: 10.29193/RMU.34.3.6.

Carhuallanqui, A., Salazar, M., & Doris, D. (2020). Efecto antimicrobiano del aceite esencial de Orégano frente a *Listeria monocytogenes* y *Staphylococcus aureus*. Journal of High Andean Research. 22(1):23-33. doi: 10.18271/ria.2020.530.

Clinical and Laboratory Standards Institute (CLSI). 2020. Performance Standards for Antimicrobial Susceptibility Testing M100-S30. 30thEd. Wayne, PA, USA.

Cruz, A., Brito, I., Sobral, M., Sousa, A., Alves, E., Andreza, R., Ferreira, S., Costa, R., Figueredo, F., Tam, T., Macêdo, R., Ribeiro, T., Leandro, L., Oliveira, C., Tintino, S., Costa, M., Aquino, P. 2016. Avaliação da atividade antibacteriana e moduladora dos extratos metanólico e hexânico da folha de *Allium cepa*. Revista Ciencias de la Salud. 14 (2): 191-200. doi: 10.12804/revsalud14.02.2016.04.

Das, S., Chourashi, R., Mukherjee, P., Kundu, S., Koley, H., Dutta, M., Mukhopadhyay, A.K., Okamoto, K., Chatterjee, N.S. 2021. Inhibition of growth and virulence of *Vibrio cholerae* by carvacrol, an essential oil component of *Origanum* spp. Journal of Applied Microbiology. 131 (3): 1147–1161. doi:10.1111/jam.15022.

Demirtaş, A., Öztürk, H., Pişkin, I. 2018. Overview of plant extracts and plant secondary metabolites as alternatives to antibiotics for modification of ruminal fermentation. Ankara Universitesi Veteriner Fakultesi Dergisi. 65: 213-217.

doi:10.1501/Vetfak_0000002849.

Dorjgochoo, A., Batbayar, A., Tsend-Ayush, A. Erdenebayar, O., Byambadorj, B., Jav,S., & Yandag, M. (2023). Detection of virulence genes of *Staphylococcus aureus* isolated from raw beef for retail sale in the markets of Ulaanbaatar city, Mongolia. BMC Microbiology. 23(1). doi: 10.1186/s12866-023-03122-2.

Eldin, A., Karima, Abdallah, H. E., Algahtani, F. D., Mohamed Tharwat Elabbasy, Suleman Atique, Ahmad, K., Mohammad, Abdallah, H. M., & Abdallah. (2023). Potential of Natural Phenolic Compounds as Antimicrobial Agents against Multidrug-Resistant *Staphylococcus aureus* in Chicken Meat. Molecules. 28(18): 6742–6742. doi: 10.3390/molecules28186742.

Enright, M.C., Day, N.P., Davies, C.E., Peacock, S.J. and Spratt, B.G. (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin- susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38. 1008–1015. doi: 10.1128/JCM.38.3.1008-1015.2000.

Franco, L.O., Samuel Teixeira Lopes, Mateus Torres Nazari, Bárbara Biduski, Vânia Zanella Pinto, Sena, Telma Elita Bertolin, & Ruschel, L. (2022). Fruit pomace as a promising source to obtain biocompounds with antibacterial activity. Critical Reviews in Food Science and Nutrition. 1–13. doi: 10.1080/10408398.2022.2103510.

Gerardi, C., Pinto, L., Baruzzi, F., & Giovinazzo, G. (2021). Comparison of Antibacterial and Antioxidant Properties of Red (cv. Negramaro) and White (cv. Fiano) Skin Pomace Extracts. Molecules. 26(19): 5918–5918. doi: 10.3390/molecules26195918.

Gerding, M., Howieson, J.G., O’Hara, G.W., Real, D., Bräu, L.

2013.Establishment and survival of the South African legume *Lessertia* spp. and rhizobia in Western Australian agricultural systems. Plant Soil. 370: 235–249. doi: 10.1007/s11104-013-1632-1.

Gnat, S., Majer-Dziedzic, B., Nowakiewicz, A., Trościańczyk, A., Ziółkowska, G., Jesionek, W., Choma, I., Dziedzic, R., Zięba, P.2017. Antimicrobial activity of some plant extracts against bacterial pathogens isolated from faeces of red deer (*Cervus elaphus*). Polish Journal of Veterinary Sciences. 20 (4): 697-706. doi: 10.1515/pjvs-2017-0087.

Goulas, V., Stavrou, K., Michael, C., Botsaris, G., Barbouti, A. 2021.The Potential of Sun-Dried Grape Pomace as a Multi- Functional Ingredient for Herbal Infusion: Effects of Brewing Parameters on Composition and Bioactivity. Antioxidants. 10 (4): 586. doi: 10.3390/antiox10040586.

Guillén, R., Carpinelli, L., Fátima Rodríguez, Castro, H.,Quíñónez, B., Campuzano, A., María Luisa Macchi, Ortellado, J., Almada, P., Grau, L., Mónica Rodríguez, Velázquez, G., Espínola, C., Samudio, G., Gómez, G., & Basualdo, W. (2016). *Staphylococcus aureus* adquiridos en la comunidad: caracterización clínica, fenotípica y genotípica de aislados en niños paraguayos. Revista Chilena de Infectologia. 33(6): 609–618. doi: 10.4067/S0716-10182020000100037.

Hao, Y., Li, J., & Shi, L. (2021). A Carvacrol-Rich Essential Oil Extracted from Oregano (*Origanum vulgare* “Hot & Spicy”) Exerts Potent Antibacterial Effects Against *Staphylococcus aureus*. Frontiers in Microbiology. 12:741861. doi: 10.3389/fmicb.2021.741861.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary

Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33, 1870–1874. doi: 10.1093/molbev/msw054.

Lee, D.-U., Yeong Jin Park, Cho Eun Kang, Cui, C.-H., Lee, D.-H., Lee, N., & Paik, H. (2022). Synergistic antimicrobial activity of ε-polylysine, chestnut extract, and cinnamon extract against *Staphylococcus aureus.* Food Science and Biotechnology. 31(5): 607–615. doi: 10.1007/s10068-022-01065-4.

Liu, B., Zhang, X., Ding, X., Wang, Y., Zhu, G. 2021. Regulatory mechanism of sub-inhibitory levels antibiotics agent in bacterial virulence. Applied Microbiology and Biotechnology.105 (9): 3495-3505. doi:10.1007/s00253-021-11291-1.

Lofa, A., Velasco, V., Gerding, M., López, M., Vallejos, D., Bonilla, A., Logue, C. 2019. Antibiotic-resistant *Staphylococcus aureus* strains of swine origin: molecular typing and susceptibility to oregano (*Origanum vulgare* L.) essential oil and maqui (*Aristotelia chilensis* (Molina) Stuntz) extract. J Appl Microbiol. 127(4):1048-1056. doi: 10.1111/jam.14393.

Luchian, C. V. Cotea, L. Vlase, A. Toiu, L. Colibaba, Răschip, I. E., G. Nadăș, A. Gheldiu, C. Tuchiluş, & L. Rotaru. (2019). Antioxidant and antimicrobial effects of grape pomace extracts. BIO Web of Conferences. 15:04006. doi:10.1051/bioconf/20191504006.

Marinho, T. A., Oliveira, M. G., Menezes-Filho, A. C. P., Castro, C. F. S., Oliveira, I. M. M., Borges, L. L., Melo-Reis, P. R., & Silva-Jr, N. J. 202. Phytochemical characterization, and antioxidant and antibacterial activities of the hydroethanolic extract of *Anadenanthera peregrina* stem bark.

Brazilian Journal of Biology. 82: e234476. doi:10.1590/1519-6984.234476.

Mirabal, G.A. 2020. Resistencia a los antimicrobianos en hospitales infantiles: perspectiva y realidad. Revista Biomedica. 31 (1): 1-2. doi:10.32776/revbiomed.v31i1.784.

Moore, D.S. 2007. The basic practice of statistics. 4th Edition. W.H. Freeman and Company, New York, USA. 560 p.

Navarrete N., Pita, E., Sánchez, R., Giraldo S., Bernal M. 2020. Actividad in vitro de los extractos etanólicos *de Lantana camara* L., *Petiveria alliacea* L. y *Lippia dulcis* T. frente a bacterias patógenas. Nova. 18 (33): 53-71. doi:10.22490/24629448.3700.

Ngamwongsatit, P., Banada, P., Panbangred, W., Bhunia, A. (2008). WST-1-based cell cytotoxicity assay as asubstitute for MTT-based assay for rapid detection of toxigenic Bacillus species using CHO cell line. J Microbiol Methods. 73:211–215. doi: 10.1016/j.mimet.2008.03.002.

Organización mundial de la salud (OMS). 2020. Inocuidad de alimentos [en línea]. < https://www.who.int/es/news-room/fact-sheets/detail/food-safety > [Consulta: 17 mayo 2021].

Parola I., Guevara, R., Feregrino, A., Reynoso, R., Pérez, I., Ocampo, R., Rojas, A., Luna, F., Tovar, E. 2021. Phenolic compounds and antioxidant activity of methanolic extracts from leaves and flowers of chilcuague (*Heliopsis longipes*, Asteraceae). Botanical Sciences. 99 (1): 149-160. doi:10.1007/s00299-022-02858-x.

Pyzik, E., Marek, A., & Hauschild, T. (2014). Characterisation of *Staphylococcus aureus* and *Staphylococcus aureus* – like strains isolated from table eggs. Journal of Veterinary Research. 58(1): 57–63. doi:10.2478/bvip-2014-0009.

Rehman, S., Mujtaba Ghauri, S., Sabri, A. N. 2016. Impact of Plant Extracts and Antibiotics on Biofilm Formation of Clinical Isolates from Otitis Media. Jundishapur journal of microbiology. 9 (1): e29483. doi: 10.5812/jjm.29483.

Rota, C., Carraminana, J., Burillo, J., Herrera, A. 2004. In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. Journal of food protection. 67(6). 1252-1256. doi: 10.4315/0362-028X-67.6.1252.

Rota, M., A. Herrera, R. Martinez, J. Sotomayor, M. Jordan. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control. 19: 681-687. doi:10.1016/J.FOODCONT.2007.07.007.

Serra, M. (2017). La resistencia microbiana en el contexto actual y la importancia del conocimiento y aplicación en la política antimicrobiana. Revista Habanera de Ciencias Médicas [online]. 16 (3): 402-419. ISSN-e 1729-519X.

Shao, K., Sun, J., Lin, Y., Zhi, H., Wang, X., Fu, Y., Xu, J., & Liu, Z. (2023). Green Synthesis and Antimicrobial Study on Functionalized Chestnut-Shell-Extract Ag Nanoparticles. Antibiotics. 12(2): 201–201.doi:10.3390/antibiotics12020201.

Singleton, Vernon L., Rudolf Orthofer, and Rosa M. Lamuela-Raventós. (1999). Analysis of totl phenols and other oxidation substrates and antioxidants by means of Folin – Ciocalteus reagent. Methods in enzymology. Vol. 299. Academis press: 152-178. doi: 10.1016/S0076-6879(99)99017-1.

Tayel, A., El-Sedfy, M., Ibrahim, A., Moussa, S. 2018. Application of *Quercus infectoria* extract as a natural antimicrobial agent for chicken egg decontamination. Revista Argentina de Microbiología. 50 (4): 391-397. doi: 10.1016/j.ram.2017.12.003.

Turchi, B., Mancini, S., Pistelli, L., Najar, B., Cerri, D., & Fratini, F. (2017). Sub-inhibitory stress with essential oil affects enterotoxins production and essential oil susceptibility in *Staphylococcus aureus*. Natural Product Research. 32(6):682-688. doi:10.1080/14786419.2017.1338284.

Vekemans, X., Beauwens, T., Lemaire, M., Roldan-Ruiz, I., 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Mol. Ecol. 11: 139–151. doi:10.1046/j.0962-1083.2001.01415.x.

Versalovic, J., Koeuth, T., Lupski, R., 1991. Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes. Nucleic Acids Res. 19: 6823–6831. doi: 10.1093/nar/19.24.6823.

Wang, X., Li, Y., Liu, S., Wang, H., Chang, X., & Zhang, J. (2023). Chestnut Shell Polyphenols Inhibit the Growth of Three Food-Spoilage Bacteria by Regulating Key Enzymes of Metabolism. Foods. 12(17): 3312–3312. doi: 10.3390/foods12173312.

Wu, X., G.R. Beecher, J.M. Holden, D.B. Haytowitz, S.E. Gebhardt,R.L. Prior. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of Agriculture and Food Chemistry. 52 (12): 4026-37. doi: 10.1021/jf049696w.

Yang, X., Zhang, J., Yu, S., Wu, Q., Guo, W., Huang, J., & Cai, S. (2016). Prevalence of Staphylococcus aureus and Methicillin-Resistant Staphylococcus aureus in Retail Ready-to-Eat Foods in China. Frontiers in Microbiology. 7. doi:10.3389/fmicb.2016.00816.

Zhu Y., Li, C., Cui, H., Lin, L. 2021. Encapsulation strategies toenhance the antibacterial properties of essential oils in food system. Food Control. 123 (2): 107856. doi: 10.1016/j.foodcont.2020.107856.

Zendejas-Manzo, Hector Avalos-Flores, & Marisela Yadira Soto-Padilla. (2014). Microbiología general de *Staphylococcus aureus*: Generalidades, patogenicidad y métodos de identificación. Revista BiomDica. 25(3): 129–143. https://doi.org/10.32776/revbiomed.v25i3.42.

Table 1. Prevalence of *Staphylococcus aureus* isolated from poultry production chain.

Type of sample	No. of samples	Positive samples of *S. aureus* (prevalence)
Animals	120	14 (3.93%)
Conventional	50	11 (22.00%) a
Cage-free	70	3 (4.29%) b
Meat	98	15 (4.21%)
Packaged	73	8 (10.96%) a
Non-packaged	25	7 (28.00%) b
Eggs	138	19 (5.35%)
Conventional	102	18 (17.65%) a
Cage-free	36	1 (2.78%) b
Total	356	48 (13.64%)

Different letters indicate significant differences (Chi-square / Fisher tests, $P \leq 0.05$).

Table 2. Antibiotic-resistance profile and inhibitory activity of plant extracts against *Staphylococcus aureus* strains from poultry production chain.

Strains	Origin	Resistance profile	Inhibition halo diameter (mm)		
			Oregano	Grape pomace	Chestnut shell
1 (skin)	Conventional	Susceptible	≥20	-	8.43 ± 0.71
2 (skin)	Cage-free	PEN-TET-CLO	≥20	-	9.59 ± 0.76
3 (egg)	Conventional	TSX-CLO-CIP	≥20	-	8.85 ± 1.94
4 (egg)	Cage-free	TSX	≥20	-	9.64 ± 1.74
5 (egg)	Conventional	Susceptible	≥20	-	12.16 ± 4.04
6 (egg)	Conventional	TET	≥20	-	9.84 ± 1.74
7 (meat	Non-packaged	PEN-OXA-FOX-TET-TSX- DA-ERY-CLIP	≥20	-	12.19 ± 2.58
8 (meat)	Non-packaged	PEN-OXA-FOX-CEF-TET- DA-ERY	≥20	-	8.97 ± 0.91

PEN: Penicillin, OXA: Oxacilin, FOX: Cefoxitin, ERY: Erythromycin, VAN: Vancomycin, GEN: Gentamicin, TSX: Trimethoprim sulfamethoxazol, CLO: Chloramphenicol, TET: Tetracycline, CEF: Ceftaroline, DA: Clindamycin, CIP: Ciprofloxacin.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts in *Staphylococcus aureus* strains from poultry production chain.

Strains	MIC		MBC	
	Oregano (%)	Chestnut shell (mg/mL)	Oregano (%)	Chestnut shell (mg/mL)
1 (skin)	0.0030	37.50	0.0625	37.5
2 (skin)	0.0015	37.50	0.0030	75.0
3 (egg)	0.0015	75.00	0.0030	150.0
4 (egg)	0.0015	37.50	0.0030	37.5
5 (egg)	0.0030	18.75	0.0030	37.5
6 (egg)	0.0015	75.00	0.0030	75.0
7 (meat	0.0015	75.00	0.0030	150.0
8 (meat)	0.0015	75.00	0.0030	75.0

Supplementary data

Capítulo 2

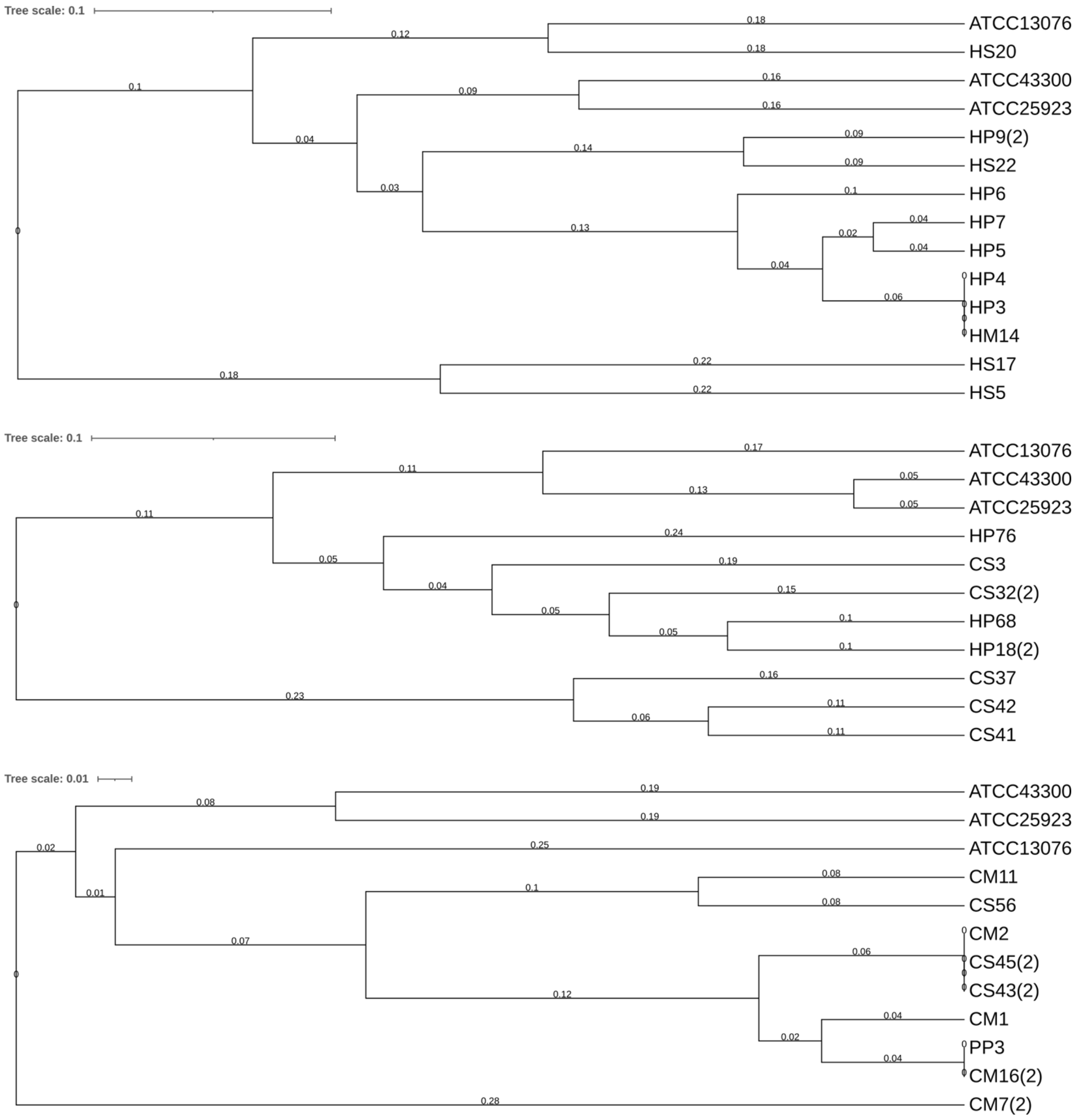
Suppl. data S1. Sample size determination.

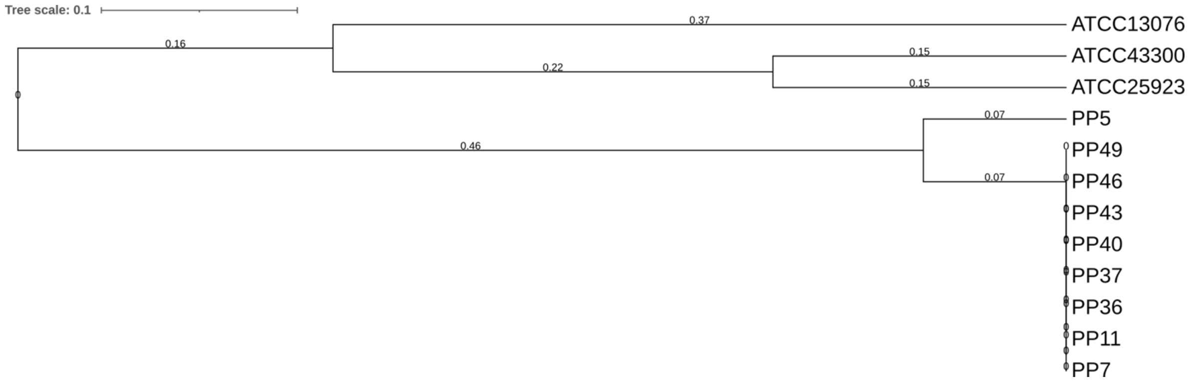
Type of sample	Sampling place	No. samples	Prevalence of antibiotic-resistant bacteria	Author
Animals (skin)	Farms	107	0.075	El-Adawy et al. (2016)
Poultry meat	Supermarkets/Butcher shops	98	0.068	Boost et al. (2013)
Eggs	Farms/Supermarkets	133	0.096	Pondit et al. (2018)
Total		≥ 338		

Supplementary data

Capítulo 2

Suppl. data S3. Dendrogram *S. aureus* (ERIC-Fingerprinting PCR).




Tree scale: 0.1
0.37
0.16
0.22
0.15
0.15
0.07
0.46
0.07
ATCC13076
ATCC43300
ATCC25923
PP5
PP49
PP46
PP43
PP40
PP37
PP36
PP11
PP7

Capitulo 3. Plant extracts for the control of antibiotic-resistant *Salmonella* spp. isolated from poultry

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Abbreviated running headline: Plant extracts against *Salmonella* from poultry

Revista: Journal of Applied Microbiology

Fecha de envío: Por enviar

Abstract

Aim: To determine the susceptibility of *Salmonella* spp. strains of poultry origin to antibiotics, and to oregano essential oil (EO) (*Origanum vulgare* L.), chestnut shell extract (*Castanea Sativa*) and grape pomace extract (*Vitis vinifera*).

Methods and Results: Samples were taken from hens and broiler chickens (cloacal swabs) (n=120), poultry meat (n=98) and eggs (n=138). *Salmonella* spp. was isolated using selective enrichment and culture method and the confirmation by PCR (identification of *hilA* gen). Strains were selected according to the genetic diversity by ERIC-fingerprinting PCR. Antibiotic and plant extracts susceptibility was determined using the disk diffusion method, and the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) by the dilution method. The total prevalence of *Salmonella* spp. was 6.53% (23/356), and only meat samples were positive, with a higher prevalence in packaged meat ($P \leq 0.05$). All selected strains were multidrug resistant (MDR). Oregano EO and chestnut shell extract had antibacterial activity against *Salmonella* spp. isolates. However, grape pomace extract did not exhibit inhibitory activity.

Conclusions: Oregano EO and chestnut shell extract have inhibitory activity against *Salmonella* spp. strains of poultry origin, with no effect with grape pomace extract.

Impact statement: This study provides information on the prevalence of *Salmonella* spp. strains in poultry and on the potential use of plant extracts to control antibiotic-resistant *Salmonella* spp. strains in the poultry supply chain.

Keywords: antimicrobial resistance (AMR), poultry, oregano essential oil (EO), chestnut shell, grape pomace

Introduction

Antimicrobial resistance (AMR) is a public health threat worldwide, resulting in 2019 in 1.27 million deaths. Antibiotic resistance arises from the misuse and overuse of antibiotics, causing susceptible bacteria death, allowing resistant ones to persist and reproduce (WHO, 2023; AGDOH, 2023). Antibiotic-resistant pathogenic bacteria show a low response to treatments, generating complications (Chis *et al*., 2022). *Salmonella* spp., is generally found in soils, water and digestive systems of animals and humans, and can interact with antibiotics, generating selective pressure in the environment. This interaction can promote genetic exchange with other microorganisms (Fernandez *et al*., 2021). The presence of *Salmonella* spp, mainly of fecal origin, in poultry meat during processing, handling, marketing and storage can cause diseases in humans (salmonellosis). *Salmonella* spp. and *E. coli* are the main bacterial agents associated with intestinal infections linked to poultry products (López *et al*., 2021).

Although it is not part of the typical bacterial flora of human skin and mucous membranes, it acts as an opportunistic pathogen, triggering various infections in humans and animals, such as gastroenteritis, typhoid fever and septicemia (Shen *et al*., 2020). During the last years, significant progress has been made in the search for alternatives to classical antibiotics in various sectors. Various natural alternatives, such as probiotics, bacteriocins, antioxidants, and plant-derived chemical compounds, have been examined in the food and veterinary industry. Among these options, plant extracts have demonstrated benefits, such as their antioxidant and antimicrobial properties in some cases (Heredia *et al*., 2022).

Studies suggest that plant phytochemicals offer a promising alternative against multidrug resistant (MDR) *Salmonella* spp. These compounds target resistance factors such as efflux pumps, membrane proteins and biofilms (Almuzaini, 2023). In addition, plant phytochemicals have economic benefits by reducing the costs of synthesis and purification of antimicrobial compounds. Their versatility in action against multiple bacterial targets may also delay the emergence of resistance (Khare *et al*., 2021).

Some plants can produce phenolic compounds as secondary metabolites in response to environmental stress or as a mechanism of defense against pathogens. In addition, upon injury or stress, plants rapidly increase the synthesis of these compounds to promote wound healing and repair (Hu *et al.,* 2022). Several types of phytochemicals, including alkaloids, phenols, coumarins, and terpenes, have been shown to be effective inhibitors against MDR pathogens, as proven in previous studies (Suganya *et al*., 2022).

The safety of poultry products is a major concern. According to a study published in Current Clinical Microbiology Reports, pre-harvest food safety interventions are crucial to control foodborne pathogens in chickens (reduce the prevalence of flocks contaminated with a specific pathogen and reduce the concentration of a pathogen in broilers belonging to contaminated flocks) (Pessoa *et al*., 2021). In addition, the poultry industry faces significant challenges, including the need to ensure the safety of poultry products following the COVID-19 outbreak (Hafez & Attia 2020).

Therefore, the aim of this study was to determine the prevalence and susceptibility of *Salmonella* spp. strains of poultry origin to antibiotics, and to plant extracts: essential oil (EO) of oregano, chestnut shell extract and grape pomace extract.

Materials and methods

Sampling

A total of 356 samples were obtained from the poultry chain (animals, eggs, meat) between December 2021 and January 2022. Samples were taken from different stages of the poultry meat and egg production chain, in region of Ñuble and región of Biobío, Chile. Sample size (Suppl. data S1) was determined using equation 1 (Moore, 2007).

$$n \geq \left(\frac{z}{m}\right)^2 \times \hat{p}\left(1 - \hat{p}\right) \qquad (1)$$

Where n is the sample size, z is the standard score in the normal distribution frequency, m corresponds to the margin of error and $\hat{p}$, is the prevalence of pathogens presenting AMR obtained in other studies. The confidence level of the interval corresponds to 95%, with z=1.96 and m= 0.05 (considering an interval no greater than 10%).

Cloacal swabs samples were collected from laying hens and broiler chicken in farms (n = 120); samples from whole and cut-up chicken meat were purchased in supermarkets, butcher shops and retail stores (meat) (n = 98); and swabs samples from eggs surface was obtained in farms, and from eggs purchased in supermarkets and retail stores) (n = 138). The legal and ethical approval was obtained from the Ethics, Bioethics and Biosecurity Committee of the University of Concepcion, Chile, prior to conducting sampling (Suppl. data S2). Samples were immediately transported at 4°C and processed.

Salmonella isolation

The isolation of *Salmonella* spp. was carried out by selective enrichment and culture method. Briefly, swab samples were immersed in 10 mL of sterile buffered peptone water (BPW), and meat samples (25 g) with MHB +6.5% NaCl (225 mL) were placed into a sterile filter bag and homogenized using a laboratory blender (BagMixer400 P; Interscience, St. Nom, France) at 8 strokes per second for a duration of 90 seconds. The enrichment was then incubated for 24 h at 35±2°C. After incubation, the enrichment was cultured on Xylose-Lysine-Deoxycholate agar (XLD) and incubated for 48 h at 35±2°C. Presumptive positive colonies (black color with red halo) were selected and streaked on trypticase soy agar (TSA) and incubated for 24 h at 35±2 °C. Presumptive *Salmonella* spp. colonies were stored at -80°C in cryotubes containing brain heart infusion broth (BHI) with 20% glycerol.

DNA Extraction

DNA from presumptive *Salmonella* spp. strains was extracted using the boiling the method proposed by Ngamwongsatit *et al*. (2008). Briefly, each strain was streaked on TSA and incubated for 24 h at 35±2°C. Then, 1 to 3 colonies were inoculated into 1 mL of trypticase soy broth (TSB) and centrifuged at 5000 x g for 2 min (Prism R™ Refrigerated MicroCentrifuge; Labnet;EE. UU.). The pellet was resuspended in 500 µL of DNA/RNAase-free H_2O and centrifuged at 5000 x g for 2 min. Subsequently, the pellet was resuspended in 100 µL of DNA/RNAase-free H_2O and kept at 100°C for 10 min (AccuBlock™ digital dry baths; Labnet;EE. UU.) followed by centrifugation at 1000 x g for 5 min. The resulting supernatant was stored at -20°C. DNA concentration was adjusted at A260/A280 = 1.8 – 2.0 (10 – 100 ng /mL).

Identification/confirmation of *Salmonella*

PCR was used to confirm *Salmonella*, identifying the *hilA* gene (transcriptional activator of invasion genes, 784 bp). The primers (sequences) were *hilA* 1 (5' CGG AAC GTT ATT TGC GCC ATG CTG AGG TAG 3') and *hilA* 2 (5' GCA TGG ATC CCC GCC GGC GAG ATT GTG 3'). To carry out the PCR reactions, a volume of 25 µL was used, including 2 µL of DNA template (<250 ng), 8.6 µL 0.7X GoTaq Master Mix (200 µM dNTPs, 3 mM MgCl2), 1 µL primers (0.5 µM) from Integrated DNA Technologies Inc. (Coralville, IA, USA). PCR conditions were adjusted following the protocol described by Velasquez *et al.* (2018) using a thermocycler (Labnet™ Thermocycler MultiGene™ OptiMax TC9610-230, Hamburg, Germany). Subsequently, 10 µL of the obtained products were loaded on 1.5% agarose gels in 1X TAE with 10 µL SafeView as DNA-intercalating dye. A molecular weight marker 100-bp ladder (Maestrogen, Inc., Las Vegas, NV, USA) was included in each gel.

Control positive-*Salmonella* ATCC 13076, control negative - *S. aureus* ATCC 25923 were included. Electrophoresis was carried out at 100 V in TAE 1X for 1.5 h. Bands were visualized using a UV transilluminator at 312 nm (BIOTOP TU1002; Shangai Bio-Tech Co. Ltd. Shangai, China).

ERIC-Fingerprinting PCR for *Salmonella*

The genetic diversity of the confirmed isolates of *Salmonella* spp. was assessed at the strain level by ERIC-PCR molecular fingerprinting using the primers Eric1R and Eric2F (ERIC1R: 5' ATGTAAGCTCCTGGGGATTCAC 3'; ERIC2F: 5' AAGTAAGTGACTGGGGTGAGCG 3') (Versalovic *et al*., 1991). Reference strains of *Salmonella enterica* subsp. *enterica* ATCC 13076 was used as positive control, while *Staphylococcus aureus* ATCC 43300 was used as negative control. Genomic DNA from reference strains was obtained using E.Z.N.A.® Tissue DNA Kit (Omega Bio-Tek) according to the manufacturer´s instructions.

The ERIC PCR mix was performed using 2.0 µL of 50 ng DNA template, 1.25 µL of forward and reverse primers 10µM, 12.5 µL of GoTaq® Colorless Master Mix (Promega) and 9.5 µL of nuclease free grade water (Promega) to total 25 µL. The reaction mixture was held at 94°C for 4 minutes, followed by 35 cycles at 94°C for 1 min, 25°C for 1 min and 72°C for 4 min with a final hold at 72°C for 5 min (Lamas *et al.,* 2016).

The PCR products were electrophoresed in 2% (w/v) agarose gels with a previous standardization of the DNA content for each sample. Each lane was charged with a mix of 15 µL of PCR product and 3 µL of loading dye supplemented with SafeView Plus DNA gel stain 20,000X (Fermelo Biotec). The marker used was 100 bp Plus DNA ladder (Maestrogen). Electrophoresis was carried out in tanks buffered with 1xTAE (40mM Tris-Acetate, 1mM EDTA, pH 8.0) at 100V for 3 h. Bands were visualized in a UV transilluminator at 320 nm.

The banding patterns were manually analyzed to scan for the presence of PCR products of specific molecular sizes using the Gerding *et al.,* (2013) protocol. A binary matrix was constructed with the scores 1 or 0 for the presence or absence of a band at each molecular size. The matrix was then analyzed with AFPL SURV version 1.0 (Vekemans *et al.,* 2002) to calculate the genetic distance among isolates. The distance matrix was then subjected to UPGMA cluster analyses using the NEIGHBOR application from the PHYLIP software package. The cladograms were visualized in MEGA7 (Kumar *et al.,* 2016) and strains were selected according to their genetic diversity (Suppl. Data S3).

Susceptibility to antibiotics

Selected *Salmonella* spp. strains by ERIC-fingerprinting were subjected to antibiotic susceptibility testing using the disk diffusion method. Briefly, 0.1 mL of a bacterial solution (0.85% saline NaCl solution, McFarland 0.5 ≈ 10^8 CFU) was streaked on Mueller-Hinton agar (MHA) using a sterile cotton swab. After placing the discs on the agar surface, the samples were incubated at 35 ± 2 °C for 24 h. For the interpretation of the results the criteria indicated by the Clinical and Laboratory Standard Institute (CLSI, 2020) was used.

Seven antibiotics covering seven different classes were examined: ampicilin, ceftaroline, gentamicin, ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, oxitetracycline.

Characterization of plant extracts

The following extracts were evaluated: oregano essential oil (EO) (R.C. Treatt & Co

Ltd, Suffolk, UK), grape pomace extract (Carmenere, Merlot and Syrah from region of Maule, Chile.) and chestnut shell extract. Quantification of total phenolic compounds content of grape pomace and chestnut shell extracts was carried out following the method of Singleton et al. (1999), a modification of the Folin-Ciocalteu method. In this procedure, the Folin-Ciocalteu reagent and a 20% (m/v) sodium carbonate solution was used. The absorbance of the samples was measured at a wavelength of 765 nm using a microplate multilector. The results obtained were expressed as Gallic Acid equivalents per gram of extract (GAE/g extract).

Susceptibility to plant extracts

Oregano EO was prepared in absolute ethanol (99.8%; Merck, Darmstadt, Germany) to obtain a concentration of 5.0%. Grape pomace extract was tested in ethanol-distilled water concentrations at a 60:40 v/v ratio. While the chestnut shell extract was prepared with ethanol to reach a concentration of 40%. Ethanol (Emsure®, Merck) was used as a control.

Plant extracts were sterilized using a 0.22 µm filter (EDLAB CA Syringe Filter). Then, the antimicrobial activity of these agents was evaluated by the disk diffusion method using sterile paper disks (Whatman No. 1, 6 mm diameter) according to the procedure described by Rota *et al.* (2008).

Bacterial suspensions were prepared in trypticase soy broth (TSB) with an optical density of 0.1 (OD_{600} = 0.1) measured in a UV/VIS spectrophotometer (BioTek Epoch Microplate Spectrophotometer, Epoch Life Science, Inc., USA). Then, a volume of 0.1 mL of this suspension was inoculated onto TSA plates. Sterile discs impregnated with 15 µL of each NA solution (oregano EO 5.0%, chestnut shell 40%, grape pomace 60/40% v/v) were placed on the previously inoculated plates, which were incubated at 35 ± 2 °C for 24 h. The inhibition criteria used for natural agent susceptibility testing (including disk diameter) were as follows: an inhibition zone ≥20 mm indicated strong inhibition; between <20 and 12 mm, moderate/slight inhibition; and <12 mm indicated lack of inhibition (Rota et al. 2008). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by the dilution method according to Rota *et al*. (2004)

Samples were inoculated on TSA agar plates and incubated at approximately 35 ± 2 °C for 24 h. The sample was then transferred from TSA agar plates to trypticase soy broth (TSB) in 2 mL Eppendorf tubes, adjusted to an optical density (OD) of 0.1. An aliquot of this solution was inoculated into each dilution (0.5%, 0.25%, 0.125%, 0.06%, 0.03%, 0.015% and 0.007%) of the prepared plant extract in triplicate (Oregano EO 5%, chestnut shell 40%, grape pomace 60/40% v/v). Ethanol solutions and blanks were used as controls to validate the results obtained. After inoculation, the sample was mixed and the initial O.D. was recorded before incubating the covered plates for 24 h at 35 ± 2 °C. After incubation, the O.D. was read again and samples with an O.D. equal to or higher than the initial one was selected and inoculated on TSA agar plates and incubated for 24 h at 35 ± 2 °C to allow growth.

Statistical analysis

The Chi-square method or Fisher's exact test was used to determine the differences in the prevalence of *Salmonella* between different type of samples taken from the poultry production chain (P≤0.05) (Moore, 2007). Differences in the sizes of inhibition zones were assessed using descriptive statistics. These criteria were based on the approach by Rota *et al*. (2008).

Results

Table 1 shows the prevalence of *Salmonella* spp. strains isolated from the poultry production chain. *Salmonella* spp. positive samples were found only in meat samples. The total prevalence of *Salmonella* spp. was 6.53% (23/356).

Three *Salmonella* spp. strains were selected according to the genetic diversity determined by the ERIC fingerprinting (Suppl. data S3). Table 2 shows antibiotic resistance profile of selected *Salmonella* spp. strains isolated from the poultry production chain.

Selected *Salmonella* spp. strains exhibited multidrug resistance (MDR), indicating their ability to resist three or more classes of antibiotics.

The compound with the highest concentration was carvacrol in oregano EO. As for grape pomace, Cabernet Sauvignon extract had the highest content of phenolic compounds (316.97 ± 22.57 mg GAE/g), followed by Carmenere (280.31 ± 8.84 mg GAE/g), and Merlot (259.75 ± 5.74 mg GAE/g). No biological activity against *Salmonella* spp. strains was observed in grape pomace extracts. A significant higher concentration of phenolic compounds was obtained with chestnut shell extract, with 181.93 ± 13.10 mg GAE/g.

The inhibitory activity of chestnut shell extract was moderate against *Salmonella* spp. strains (Table 3).

Oregano essential oil exhibited inhibitory activity against all *Salmonella* spp. strains with halo diameters ≥20 mm, classifying as strong inhibitory activity (Table 3).

Both MIC and MBC of oregano EO in the three *Salmonella* strains evaluated were 0.0015 %, as indicated in Table 3.

Chestnut shell MIC ranged from 37.5 to 75 mg/mL, while CMB ranged from 75 to 150 mg/mL, as detailed in Table 3.

Discussion

In this study, the prevalence of *Salmonella* spp. in poultry was 6.53%. These positive samples were distributed into 22 isolates from packaged meat, 1 isolate from unpackaged meat, and no positive samples from animals or eggs. Among these samples confirmed as Salmonella, only three genetically distinct strains were identified by ERIC fingerprinting analysis. These results highlight the presence of *Salmonella* spp. in the poultry chain, raising significant concerns in terms of food safety and public health. The contamination of packaged meat could have occurred at stages prior to packaging, such as during slaughtering, meat handling or due to inadequate storage and transportation conditions. During handling, meat may be exposed to various sources of contamination, such as contaminated equipment, surfaces, or hands. Additionally, if proper hygiene and temperature control conditions are not maintained during storage and transportation of the products, there is a significant risk of bacterial proliferation, including that of *Salmonella* spp.

These inadequate conditions could allow pathogens present in the environment to contaminate the meat, there by contributing to the presence of *Salmonella* spp. in the samples of packaged meat.

There are domestic and international legislation, that guarantee food safety in poultry production. According to National Health Service (2016) the Food Sanitary Regulation, in raw poultry meat, a maximum of up to 10^7 CFU (Colony Forming Units) per gram is allowed. According to the FAO and WHO (2023), it is suggested that no single measure is sufficient to effectively control *Salmonella* spp. in broilers and poultry meat. The importance of multifaceted control strategies is emphasized. These strategies, supported by the revised Codex Guidelines (CXG 78-2011), are key to ensuring the safety of poultry products.

It is relevant to note that other studies have identified *Salmonella* spp. in different food categories. For example, in one study (Corredor *et al.,* 2021), *Salmonella* spp. prevalence of 0.1%, 0.2%, 13.7%, 0.1%, and 0% was reported for fruits, leafy vegetables, ready-to-eat (RTE) salad-related mixed vegetables, root vegetables, and tomatoes, respectively, and in chicken meat, 17.41%. Also, this study, identified 14 different serotypes of *Salmonella* in chicken meat, the most common being *S. paratyphi*, *S. hvittingfoss* and *S. muenster* (López *et al*., 2023).

Regarding eggs, a study in Bogota, Colombia, found a 9.4% prevalence of *Salmonella.* In addition, significant variability in the prevalence of *Salmonella* in eggs has been observed, with studies in Uruguay, India and elsewhere reporting values ranging from 5.5% to 9.4% (Castañeda *et al*., 2017). These results highlight the importance of considering various food categories when assessing the presence of *Salmonella* spp. in the food chain.

As for eggs, the absence of *Salmonella* on their surface can be due to several reasons. Firstly, Salmonella is not a pathogen of dry environments (Shaji *et al*., 2023), which could hinder its survival on the surface of eggs. Additionally, eggs possess natural antimicrobial compounds that can inhibit the growth of *Salmonella* (Whiley and Ross, 2015; Ricke *et al*., 2018). These combined factors could explain why *Salmonella* spp. was not found on the surface of the eggs in this study.

In the case of animals, especially chickens, the absence of *Salmonella* spp. can be attributed to effective management measures implemented in the farms to control this disease. *Salmonella* is a significant disease in chickens, and therefore, rigorous protocols have been established for its management in farms (Shaji *et al*., 2023). This suggests that the sampling locations are well managed, which could explain the absence of *Salmonella* in the animals. In addition, in this study, it is important to note that *Salmonella* was not detected in the cloaca, the section that was sampled. While it is true that Salmonella can colonize various parts of the gastrointestinal tract of birds, including more internal sections, it's important to note that the cloaca is considered a representative sampling location for the detection of *Salmonella* in birds. This is because it serves as one of the main excretion routes of the bacterium. In addition, this study identified three multidrug-resistant (MDR) strains within the poultry chain. These strains, detected in meat samples, exhibited resistance to three or more types of antibiotics, highlighting the risk of multi-resistance in the poultry industry. Other studies have also identified the presence of multidrug-resistant *Salmonella* strains in the poultry supply chain. In evaluations of antibiotic resistance in both chickens and pigs, significant rates of MDR were detected in *Salmonella* spp. isolated from chickens, reaching 81.1%. This resistance included sulfisoxazole (76.1%), tetracycline (75.3%), ampicillin (48.0%), and ofloxacin (44.7%) (Castro *et al.,* 2020). Similarly, a study conducted in Ethiopia found that 96.77% of *Salmonella* strains isolated from poultry products exhibited antimicrobial resistance (Asefa and Duga, 2022). These findings corroborate the concerning prevalence of MDR observed in *Salmonella* spp. isolated from chickens, with a rate of 73.2% multidrug resistance (Rodríguez, 2019).

Interest in natural by-products as antimicrobial agents is increasing. Despite the presence of phenolic compounds in grape pomace, no antimicrobial effect was observed. This viticultural by-product is characterized as a rich source of flavonoid phenols, such as anthocyanins, flavanols, flavonols and tannins, as well as non-flavonoid phenols, including phenolic acids, according to the classification proposed by Ramirez and DeWitt (2014). Although the literature highlights the antibacterial potential of grape pomace extracts against various foodborne pathogenic bacteria (Sanhueza *et al*., 2014), several studies indicate an absence of biological activity against Gram-negative bacteria. For example, Filocamo *et al*. (2015) demonstrated the efficacy of white grape juice extracts against various Gram-positive bacteria; however, these extracts had no impact on the growth of Gram-negative bacteria, including *S. typhi.* Similar results were reported by Xu *et al*. (2015) when evaluating the antibacterial activity of grape pomace. All extracts exhibited antibacterial activity against *L. monocytogenes* and *S. aureus* (gram-positive bacteria), but no antibacterial activity was detected against *E. coli* O157:H7 and *S. typhimurium* (gram-negative bacteria). This may be attributed to the cell membrane composition of gram-negative bacteria. They possess an additional protective outer membrane, which might render them more resilient to phenolic compounds (Tirado *et al.*, 2021).

In addition, phenolic compounds have membrane-active properties against bacteria, causing leakage of cellular constituents, including nucleic acids, proteins and inorganic ions such as potassium or phosphate (Lobiuc *et al*., 2023). However, this activity may be less effective in gram-negative bacteria due to the presence of lipopolysaccharides in their membrane, which may repel or slow down the interaction with polyphenols (Tirado *et al*., 2021). The complex composition of grape pomace, which includes tannins, anthocyanins, catechins, procyanidins, flavonol glycosides, phenolic acids, stilbenes, among others, hinders to understand the precise mechanism of microorganism inhibition. In addition, the variability in the distribution of these antimicrobial compounds among parts of the pomace, such as skin, seeds and blends, also contributes to the lack of clarity in the inhibitory mechanism (Hassan *et al*., 2019).

Chestnut shells harbor beneficial molecules such as phenols, flavonoids, triterpenes, sugars and tannins. The brown pigment in these shells has antioxidant, antibacterial and anti-inflammatory properties, resulting in a valuable source of bioactive compounds (Shao *et al*., 2023). Chestnut (*Castanea sativa*) shell extracts have been reported to possess antimicrobial activity against various bacterial species, including both Gram-positive and Gram-negative foodborne bacteria. Sensitive strains include *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella Typhimurium* (Lee *et al*., 2016). Other authors, such as Rodrigues *et al*. (2023), have reported positive results in relation to *Salmonella enterica*. In their research, MIC values ranging from 1.25 to 10 mg/mL, and MBC in the range of 10 to <10 mg/mL were reported. In this study, the antimicrobial activity of chestnut shell extract was assessed, with MIC ranged from 37.5 to 75 mg/mL, and the MBC ranged from 75 to 150 mg/mL. These results suggest a significant potential of chestnut shell extract as antimicrobial agent. The presence of phenolic acids in chestnut shells could be responsible for these observed antimicrobial effects.

Previous studies have shown that phenolic acids, in particular p-coumaric acid, exhibit antibacterial properties against Gram-negative bacteria. Mechanisms such as DNA binding or modification of cell membrane permeability have been identified as possible contributors to this antibacterial activity (Silva *et al*., 2020).

This study also considers the importance of the circular economy in agro-industrial waste management. In this context, the use of chestnut shell and grape pomace, both agro-industrial by-products, for the extraction of antimicrobial compounds is an example of how waste has a new use in line with the principles of the circular economy (Khanna *et al*., 2022).

These findings support existing evidence and underscore the promising ability of chestnut shell extract against Gram-negative bacteria. These results open the door for future research to better understand the underlying mechanisms and explore potential applications in the prevention and control of bacterial infections, especially those transmitted by food.

Oregano EO, is notable for its predominant content of carvacrol and thymol, with precursors such as p-cymene and γ-terpinene in smaller proportions (Sakkas and Papadopoulou, 2017). In this study, carvacrol chemotype was used. The strong antimicrobial activity of oregano EO against pathogenic bacteria is due to the abundance of phenolic compounds, including carvacrol, eugenol and thymol (Leyva *et al*., 2017). These specific compounds have been shown to possess significant antimicrobial properties, being fundamental to the efficacy of the essential oil against various microorganisms.

In this study, the MIC and the MBC of oregano EO was 0.0015 % in all *Salmonella* spp. strains. Other studies, such as the one conducted by Luna-Solorza *et al* (2023), report a MIC of 0.250 mg/mL and an MBC of 0.300 mg/mL in *S. typhirium* strains, confirming the antimicrobial potential of oregano EO.

Liu *et al*. (2022) explain that carvacrol and thymol functional groups influence hydrophobicity and antimicrobial activity by altering cell membrane permeability, thus triggering cell death in a way. These findings support the understanding of how specific components of oregano EO contribute to its antimicrobial efficacy in this study.

Conclusions

Salmonella spp. and antibiotic-resistant strains are present in the poultry production chain.

Plant extracts have an antimicrobial effect against antibiotic-resistant *Salmonella* spp. isolated in the poultry production chain, such as oregano EO with a high inhibitory activity, and chestnut shell extract with a moderately inhibitory activity on some strains.

Funding

This work was supported by the Research Project VRID No. 2021000173INV.

Conflict of Interest

No conflict of interest declared.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

References

Almuzaini, A. M. 2023. Phytochemicals: potential alternative strategy to fight *Salmonella enterica* serovar *Typhimurium*. Frontiers in Veterinary Science. 10. doi: 10.3389/fvets.2023.1188752.

Asefa, I., Duga T. 2022. Prevalence and Antimicrobial Resistance of *Salmonella* in Poultry Products in Central Ethiopia. Vet Med Int. 2022:8625636. doi: 10.1155/2022/8625636.

Australian Government Department of Health. 2023. Antimicrobial resistance [en línea]. < https://www.amr.gov.au/about-amr/what-causes-amr > [Consulta: 10 enero 2024].

Castañeda, R.,Pulido, A., Mendoza, M., Carrascal, A., & Sandoval, K. (2017). Detección e identificación de *Salmonella* spp. en huevos para consumo humano, provenientes de diferentes localidades de Bogotá, Colombia, 2015. Infectio. 21(3). doi: 10.22354/in.v21i3.672.

Castro, R. Herrera, M, Rodríguez, R., Rondón, I.2020. Antibiotic resistance in *Salmonella* spp. isolated from poultry: A global overview. Vet World. 13(10):2070-2084. doi: 10.14202/vetworld.2020.2070-2084.

Chiș, A., Rus, L., Morgovan, C., Arseniu, A., Frum, A., Vonica-Țincu. A., Gligor, F., Mureșan, L., Dobrea, C. 2022. Microbial Resistance to Antibiotics and Effective Antibiotherapy. Biomedicines. 10(5):1121. doi: 10.3390/biomedicines10051121.

Clinical and Laboratory Standards Institute (CLSI). 2020. Performance Standards for Antimicrobial Susceptibility. Testing M100-S30. 30thEd. Wayne, PA, USA.

Corredor, D., García-Pinilla, S., & Carla María Blanco-Lizarazo. 2021. Systematic Review and Meta-analysis: *Salmonella* spp. prevalence in vegetables and fruits. World Journal of Microbiology & Biotechnology. 37(3). doi: 10.1007/s11274-021-03012-7.

FAO & WHO. 2023. Measures for the control of non-typhoidal *Salmonella spp.* in poultry meat – Meetingreport. Microbiological Risk Assessment Series, No. 45. Rome. ISBN: 978-92-4-007882-6.

Fernandez, E., Bolívar-Anillo, H., Hoyos, C., Carrillo, L., Serrano, M., & Ezzanad, A. (2021). Antibiotic resistance: The role of man, animals and the environment. Salud. 36(1): 298–324. doi:10.14482/sun.36.1.615.

Filocamo, A., Bisignano, C., Mandalari, G., & Navarra, M. (2015). In Vitro Antimicrobial Activity and Effect on Biofilm Production of a White Grape Juice (*Vitis vinifera*) Extract. Evidence-Based Complementary and Alternative Medicine. 2015: 1–5. doi: 10.1155/2015/856243.

Gerding, M., Howieson, J.G., O’Hara, G.W., Real, D., Bräu, L., 2013. Establishment and survival of the South African legume *Lessertia* spp. and rhizobia in Western Australian agricultural systems. Plant Soil 370, 235–249.

doi:10.1007/s11104-013-1632-1.

Hafez, H. M., & Attia, Y. A. (2020). Challenges to the Poultry Industry: Current Perspectives and Strategic Future After the COVID-19 Outbreak. Frontiers in Veterinary Science.7. doi: 10.3389/fvets.2020.00516.

Hassan, Y., Kosir, V., Yin, X., Ross, K., and Diarra, M. 2019. Journal of Agricultural and Food Chemistry. 67 (35): 9705-9718. doi: 10.1021/acs.jafc.9b02861.

Heredia, P. Y., García-Baldenegro, C. V., Santos-Espinosa, A. de I., Manzanarez-Quin, C. G., Valdez-Domínguez, R. D., Ibarra-Zazueta, C., Osuna-Chávez, R. F., Rueda-Puente, E. O., Hernández-Moreno, C. G., Barrales-Heredia, S. M., & Sosa-Castañeda, J. (2022). Perfil fitoquímico, actividad antimicrobiana y antioxidante de extractos de *Gnaphalium oxyphyllum* y *Euphorbia maculata* nativas de Sonora, México. Revista Mexicana de Ciencias Pecuarias. 13(4): 928–942. doi: 10.22319/rmcp.v13i4.6042.

Hu, W., Sarengaowa, Guan, Y., & Feng, K. (2022). Biosynthesis of Phenolic Compounds and Antioxidant Activity in Fresh-Cut Fruits and Vegetables. Frontiers in Microbiology. 13. doi: 10.3389/fmicb.2022.906069.

Khanna, M., Gusmerotti, N. M., & Frey, M. (2022). The Relevance of the Circular Economy for Climate Change: An Exploration through the Theory of Change Approach. Sustainability, 14(7), 3991. doi:10.3390/su14073991.

Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z., Liu, Z., & Kumar, V. (2021). Exploring Phytochemicals for Combating Antibiotic Resistance in Microbial Pathogens. Frontiers in Pharmacology. 12. doi:

10.3389/fphar.2021.720726.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol. Biol. Evol. 33. 1870–1874. doi: 10.1093/molbev/msw054.

Lamas, A., Fernandez-No, I., Miranda, J., Vázquez, B., Cepeda, A., Franco, C., 2016. Prevalence, molecular characterization and antimicrobial resistance of *Salmonella* serovars isolated from northwestern Spanish broiler flocks (2011-2015). Poult. Sci. 95: 2097–2105. doi: 10.3382/ps/pew150.

Lee, N., Byeong Su Jung, Da Som Na, Hwan Hee Yu, Kim, J.-S., & Paik, H. 2016. The impact of antimicrobial effect of chestnut. inner shell extracts against *Campylobacter jejuni* in chicken meat. LWT. 65: 746–750. doi.org/10.1016/j.lwt.2015.09.004.

Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, Heredia JB. Essential Oils of Oregano: Biological Activity beyond Their Antimicrobial Properties. Molecules. 2017 Jun 14;22(6):989. doi: 10.3390/molecules22060989.

Liu, H., Whitehouse, C. A., & Li, B. (2018). Presence and Persistence of *Salmonella* in Water: The Impact on Microbial Quality of Water and Food Safety. Frontiers in Public Health. 6. doi.org/10.3389/fpubh.2018.00159.

Liu, Y., Yan, Y., Dong, P., Ni, L., Luo, X., Zhang, Y., & Zhu, L. 2022. Inhibitory effects of clove and oregano essential oils on biofilm formation of *Salmonella* Derby isolated from beef processing plant. LWT. 162: 113486–113486. doi.org/10.1016/j.lwt.2022.113486.

López, V., Guerrier, L., Elorza, V., Krüger, A., Colello, R., Medici, S. K., Espinosa, M., Casado, P., Recavarren, M. I., & Keller, L. (2021). Resistencia a antimicrobianos en bacterias aisladas en la cadena de producción avícola. InVet. 23(2): 01-08. ISSN 1668-3498 (en línea).

López, A., Burgos, T., Vanegas, M., Álvarez, Z., Mendez, Y., & Quinteros, E. 2023. Factores asociados con la contaminación microbiológica en la carne de pollo comercializada en El Salvador. Revista Peruana de Medicina Experimental Y Salud Pública. 25–33. doi.org/10.17843/rpmesp.2023.401.12100.

Lobiuc, A., Pavăl, N., Mangalagiu, I., Gheorghiță, R., Teliban, G., Amăriucăi-Mantu, D., Stoleru, V. 2023. Future Antimicrobials: Natural and Functionalized Phenolics. Molecules. 28(3):1114. doi: 10.3390/molecules28031114.

Luna, J., Ayala, J., Cruz, M., González, G., Bernal, A., Gutiérrez, M., & Silva, B. 2023. Oregano Essential Oil versus Conventional Disinfectants against *Salmonella Typhimurium and Escherichia coli* O157:H7 Biofilms and Damage to Stainless-Steel Surfaces. Pathogens. 12(10): 1245–1245. doi: 10.3390/pathogens12101245.

Moore, D.S. 2007. The basic practice of statistics. 4th Edition. W.H. Freeman and Company, New York, USA. 560 p. National Health Service. 2016. Reglamento sanitario de los alimentos. DTO. N° 977/96. Chile.

Ngamwongsatit, P., Banada, P., Panbangred, W., Bhunia, A. 2008. WST-1-based cell cytotoxicity assay as a substitute for MTT-based assay for rapid detection of toxigenic *Bacillus* species using CHO cell line. J Microbiol Methods. 73:211–215. doi: 10.1016/j.mimet.2008.03.002.

Pérez, A., García, B., & López, C. (2018). Biological Approaches for Disease Control in Aquaculture: Advantages, Limitations and Challenges. Trends in Microbiology. 26(11): 896–903. doi: 10.1016/j.tim.2018.05.002.

Pessoa, J., Rodrigues, M., Truls Nesbakken, & Meemken, D. (2021). Assessment of the Effectiveness of Pre-harvest Meat Safety Interventions to Control Foodborne Pathogens in Broilers: A Systematic Review. Current Clinical Microbiology Reports. 8(2). 21–30. doi:10.1007/s40588-021-00161-z.

Ramirez, L., and C. DeWitt, A. 2014. Analysis of phenolic compounds in commercial dried grape pomace by high-performance liquid chromatography electrospray ionization mass spectrometry. Food Sci. Nut. 2:470–477. doi: 10.1002/fsn3.136.

Ricke, S. C., Dawoud, T. M., Shi, Z., Pravin Kaldhone, & Kwon, Y. M. (2018). Foodborne *Salmonella* in Laying Hens and Egg Production. Elsevier EBooks, 153–171. doi: 10.1016/b978-0-12-811835-1.00009-9.

Rodrigues, D., Veríssimo, L., Finimundy, T., Rodrigues, J., Oliveira, I., Gonçalves, J., Fernandes, I., Barros, L., Heleno, S., Calhelha, R.2023. Chemical and Bioactive Screening of Green Polyphenol-Rich Extracts from Chestnut By-Products: An Approach to Guide the Sustainable Production of High-Added Value Ingredients. Foods. 12(13):2596. doi: 10.3390/foods12132596.

Rodríguez, E. 2019. Alarm as antimicrobial resistance surges among chickens, pigs and cattle.Nature. doi: 10.1038/d41586-019-02861-5.

Rota, C., Carraminana, J., Burillo, J., Herrera, A. 2004. In vitro antimicrobial activity of essential oils from aromatic plants against selected foodborne pathogens. Journal of food protection. 67(6). 1252-1256. doi: 10.4315/0362-028X-67.6.1252.

Rota, M., A. Herrera, R. Martinez, J. Sotomayor, M. Jordan. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control. 19: 681-687. doi:10.1016/j.foodcont.2007.07.007

Sakkas, H., & C. Papadopoulou. 2017. Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. Journal of Microbiology and Biotechnology. 27(3): 429–438. doi: 10.4014/jmb.1608.08024.

Sanhueza, L., Tello, M. D., Vivanco, M., Mendoza, L., & Wilkens, M. (2014). Relation between Antibacterial Activity against Food Transmitted Pathogens and Total Phenolic Compounds in Grape Pomace Extracts from Cabernet Sauvignon and Syrah Varieties. 04(05): 225– 232. doi:10.4236/aim.2014.45029

Shaji S, Selvaraj RK, Shanmugasundaram R. *Salmonella* Infection in Poultry: A Review on the Pathogen and Control Strategies. Microorganisms. 2023 Nov 20;11(11):2814. doi: 10.3390/microorganisms11112814.

Shao, K., Sun, J., Lin, Y., Zhi, H., Wang, X., Fu, Y., Xu, J., & Liu, Z. 2023. Green Synthesis and Antimicrobial Study on Functionalized Chestnut-Shell-Extract Ag Nanoparticles. Antibiotics. 12(2): 201–201. doi: 10.3390/antibiotics12020201.

Shen, H., Chen, H., Ou, Y., Huang, T., Chen, S., Zhou, L., Zhang, J., Hu, Q., Zhou, Y., & Ma, W. (2020). Prevalence, serotypes, and antimicrobial resistance of *Salmonella* isolates from patients with diarrhea in Shenzhen, China. BMC Microbiology. 20(1). doi: 10.1186/s12866-020-01886-5.

Silva, V., Falco, V., Dias, M., Barros, L., Silva, A., Capita, R., Alonso-Calleja, C., Amaral, J., Igrejas, G., Ferreira, I., Poeta, P. 2020. Evaluation of the Phenolic Profile of *Castanea sativa* Mill. By-Products and Their Antioxidant and Antimicrobial Activity against Multiresistant Bacteria. Antioxidants (Basel). 9(1):87. doi: 10.3390/antiox9010087.

Singleton, Vernon L., Rudolf Orthofer, and Rosa M. Lamuela-Raventós. 1999. Analysis of totl phenols and other oxidation substrates and antioxidants by means of Folin – Ciocalteus reagent. Methods in enzymology. Vol. 299. Academis press: 152-178.

Suganya, T., Packiavathy, I., Aseervatham, G., Carmona, A., Rashmi, V., Mariappan, S., Devi, N., Ananth, D. 2022. Tackling Multiple-Drug-Resistant Bacteria With Conventional and Complex Phytochemicals. Front Cell Infect Microbiol. 12:883839. doi: 10.3389/fcimb.2022.883839.

Tirado, V., Atoche, S., & Hernández, E. (2021). Phenolic compounds of mango (*Mangifera indica*) by-products: Antioxidant and antimicrobial potential, use in disease prevention and food industry, methods of extraction and microencapsulation. Scientia Agropecuaria. 12(2): 283–293. doi: 10.17268/sci.agropecu.2021.031.

Vekemans, X., Beauwens, T., Lemaire, M., Roldan-Ruiz, I. 2002. Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. Mol. Ecol. 11: 139–151. doi:10.1046/j.0962-

1083.2001.01415.x.

Velasquez, C. G., Macklin, K., Kumar, S., & Singh, M. (2018). Prevalence and antimicrobial resistance patterns of Salmonella isolated from poultry farms in southeastern United States.Poultry Science 97(6). doi:10.3382/ps/pex449

Versalovic, J., Koeuth, T., Lupski, R., 1991. Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes. Nucleic Acids Res. 19, 6823–6831. doi:10.1093/nar/19.24.6823

Whiley H, Ross K. Salmonella and eggs: from production to plate. Int J Environ Res Public Health. 2015 Feb 26;12(3):2543-56. doi: 10.3390/ijerph120302543.

World health organization (WHO). (2023). Resistencia a los antimicrobianos. [en línea]. < https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance> [Consulta: 10 enero 2024].

Xu, Y., Burton, S., Kim, C., & Sismour, E. (2015). Phenolic compounds, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties. Europe PMC (PubMed Central). 4(1): 125–133. doi: 10.1002/fsn3.264.

Table 4. Prevalence of *Salmonella* spp. isolated from poultry production chain.

Type of sample	No. of samples	Positive samples of *Salmonella* (prevalence)
Animals	120	0
Conventional	50	0
Cage-free	70	0
Meat	98	23 (23.47%)
Packaged	73	22 (30.14%) a
Non-packaged	25	1 (4.0%) b
Eggs	138	0
Conventional	102	0
Cage-free	36	0
Total	356	23 (6.53%)

Different letters indicate significant differences (Chi-square / Fisher tests, $P \leq 0.05$).

Table 5. Antibiotic-resistance profile and inhibitory activity of plant extracts against *Salmonella* strains from poultry production chain.

Strains	Origin	Resistance profile	Inhibition halo diameter (mm)		
			Oregano	Grape pomace	Chestnut shell
1 (skin)	Packaged	CEF-AMP-CIP-TET-CLO	≥20	-	-
2 (skin)	Packaged	CEF-TSX-AMP-CIP-TET-CLO	≥20	-	9.69±1.87
3 (egg)	Non-packaged	CEF-TSX-AMP-TET-CLO	≥20	-	8.39±1.09

CEF - Ceftaroline, TSX - Trimethoprim sulfamethoxazole, AMP - Ampicillin, GEN - Gentamicin, CIP – Ciprofloxacin, TET - Tetracycline, CLO - Chloramphenicol.

Table 6. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts in *Salmonella* strains from poultry production chain.

Strains	MIC		MBC	
	Oregano (%)	Chestnut shell (mg/mL)	Oregano (%)	Chestnut shell (mg/mL)
1 (skin)	0.0015	75.00	0.0015	150
2 (skin)	0.0015	75.00	0.0015	75.0
3 (egg)	0.0015	37.50	0.0015	75.0

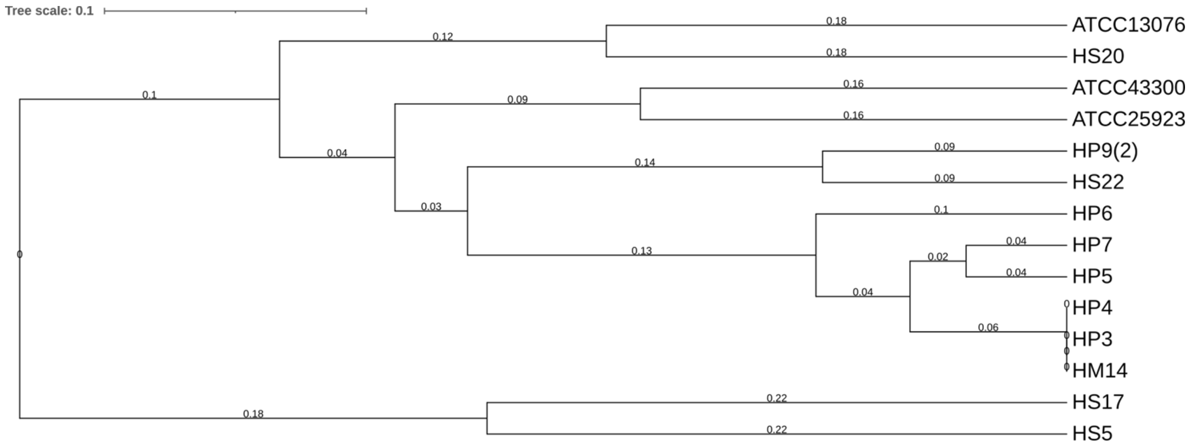
Supplementary data

Capítulo 3

Suppl. data S1. Sample size determination.

Type of sample	Sampling place	No. samples	Prevalence of antibiotic-resistant bacteria	Author
Animals (skin)	Farms	107	0.075	El-Adawy et al. (2016)
Poultry meat	Supermarkets/Butcher shops	98	0.068	Boost et al. (2013)
Eggs	Farms/Supermarkets	133	0.096	Pondit et al. (2018)
Total		≥ 338		

Suppl. data S3. Dendrogram *Salmonella* (ERIC-Fingerprinting PCR).



Conclusiones Generales

Se ha identificado la presencia de cepas de *S. aureus* y *Salmonella* spp., incluyendo aquellas resistentes a antibióticos, en diversas etapas de la cadena de producción avícola, lo que sugiere la existencia de fuentes de contaminación.

Existen extractos vegetales, como el aceite esencial de orégano y el extracto de cáscara de castaña que presentan un efecto antimicrobiano contra estas cepas resistentes. Específicamente, el aceite de orégano demostró una alta actividad inhibitoria, mientras que el extracto de cáscara de castaña mostró una actividad moderadamente inhibidora en algunas cepas.

Estos hallazgos resaltan el potencial de los extractos vegetales como alternativas eficaces para el control y propagación de patógenos resistentes a antibióticos en la industria avícola.