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Second Cycle Degree (MSc)

In Food and Health

"Colloidal suspension of polyphenolic nanoparticles as a novel strategy to combat antimicrobial resistance"

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Abbreviations

ESBL: Extended-spectrum β-lactamases

NPs: Nanoparticles

WHO: World Health Organization

MRSA: Methicillin-resistant *Staphylococcus aureus*

AMR: Antimicrobial Resistance

MDR: Multidrug resistant

ROS: Reactive oxygen species

AgNPs: Silver nanoparticles

AuNPs: Gold nanoparticles

CuO NPs: Copper oxide nanoparticles

IONPs: Iron oxide nanoparticles

MRI: Magnetic Resonance Imagining

EGCG: Epigallocatechin gallate

O.D.: Optical density

MIC: Minimum Inhibitory Concentration

CV: Coefficient of variation

BHI: Brain Heart Infusion

BA: Blood Agar

MHA: Muller Hinton Agar

VIS: Visible

UV: Ultraviolet

IR: Infrared

CFU: Colony Forming Unit

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Abstract

As anticipated and feared for a long time, antimicrobial resistance has been worsening, and nowadays many bacterial infections cannot be controlled by a simple antibiotic administration. A new and innovative approach to combat this global threat is necessary. The resistance to antimicrobials spreads between animals, the environment, and humans which makes it even more challenging to deal with. A One Health approach must be adopted and improved. Polyphenolic compounds have been explored due to their potential ability to inhibit microbial growth; however, some downsides have been connected to their use, such as low water solubility, bioavailability, and instability. Existing shortcomings of polyphenols could be overcome by implementing a nano-based approach, that could result in enhanced bioavailability, controlled drug release and targeting, and lower degradation.

In this project, the efficacy of polyphenolic nanoparticles as a potential alternative to conventional antimicrobials was investigated. A stable colloidal suspension consisting of polyphenolic nanoparticles was produced by top-down synthesis from a waste product of the wood industry. A panel of six bacteria (i.e., *Escherichia coli*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Streptococcus suis*) known for their zoonotic and resistant potential were included in this study. Minimum Inhibitory Concentration (MIC) was performed to assess the compound's antimicrobial activity. Nanoparticle dilutions with a concentration ranging from 15 mg/L to 0.46875 mg/L were tested against 5 log CFU/mL of the bacteria inoculum. The optical density (OD_{600}) was measured, and a threshold of < 0.05 absorbance was adopted to determine the minimum inhibitory concentration. Our results indicated that these nanoparticles showed different effects based on the specific bacteria tested, effectively inhibiting microbial growth at notably low concentrations of *M. haemolytica* (3.75 mg/L) and *P. multocida* (15 mg/L).

Polyphenolic nanoparticles tested demonstrate potent antimicrobial capabilities, suggesting promising applications in livestock production. By replacing conventional antimicrobials, they could mitigate the prevalence of antimicrobial resistance.

Keywords: antimicrobial resistance, pathogens, nanoparticles, polyphenolic compounds

Riassunto

Come anticipato e temuto da tempo, la resistenza antimicrobica è andata peggiorando e oggi molte infezioni batteriche non possono essere controllate con una semplice somministrazione di antibiotici. È necessario un approccio nuovo e innovativo per combattere questa minaccia globale. La resistenza agli antimicrobici si diffonde tra gli animali, l'ambiente e gli esseri umani, il che rende ancora più difficile affrontarla. L'approccio One Health deve essere adottato e migliorato. I composti polifenolici sono stati esplorati per la loro potenziale capacità di inibire la crescita microbica; tuttavia, alcuni aspetti negativi sono stati collegati al loro utilizzo, come la bassa solubilità in acqua, la biodisponibilità e l'instabilità. Le carenze esistenti dei polifenoli potrebbero essere superate implementando un approccio nanometrico, che potrebbe comportare una maggiore biodisponibilità, un rilascio e un targeting controllati dei farmaci e una minore degradazione.

In questo progetto è stata studiata l'efficacia delle nanoparticelle polifenoliche come potenziale alternativa agli antimicrobici convenzionali. Una sospensione colloidale stabile costituita da nanoparticelle polifenoliche è stata prodotta mediante sintesi top-down da un prodotto di scarto dell'industria del legno. In questo studio è stato incluso un gruppo di sei batteri (ovvero *Escherichia coli*, *Mannheimia haemolytica*, *Pasteurella multocida*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Streptococcus suis*) noti per il loro potenziale zoonotico e resistente. È stata eseguita la concentrazione minima inibente (MIC) per valutare l'attività antimicrobica del composto. Diluizioni di nanoparticelle con una concentrazione compresa tra 15 mg/L e 0.46875 mg/L sono state testate rispetto a 5 log CFU/mL dell'inoculo batterico. È stata misurata la densità ottica (OD_{600}) ed è stata adottata una soglia di assorbimento < 0.05 per determinare la concentrazione inibente minima.

I nostri risultati hanno indicato che queste nanoparticelle hanno mostrato effetti diversi in base ai batteri specifici testati, inibendo efficacemente la crescita microbica a concentrazioni particolarmente basse di *M. haemolytica* (3.75 mg/L) e *P. multocida* (15 mg/L).

Le nanoparticelle polifenoliche testate dimostrano potenti capacità antimicrobiche, suggerendo applicazioni promettenti nella produzione zootecnica. Sostituendo gli antimicrobici convenzionali, potrebbero mitigare la prevalenza della resistenza antimicrobica.

Parole chiave: resistenza antimicrobica, agenti patogeni, nanoparticelle, composti polifenolici

1 Introduction

1.1 Antimicrobial resistance

1.1.1 Introduction to Antimicrobial Resistance

Antibiotics are bioactive compounds able to inhibit microbial growth at low concentrations or induce bactericidal actions. These agents are naturally synthesized by certain bacteria and can also be produced synthetically (Malczak & Gajda, 2023).

In 1900, infectious diseases used to be among the leading causes of fatalities. A way to combat bacterial infections was desperately needed, and in 1910 first antimicrobial drug was discovered, Salvarsan (Himanshu et al., 2023). This was followed by Alexander Fleming's discovery of penicillin in 1928, which marked the origin of antibiotic therapy (Malczak & Gajda, 2023). Antibiotics have been effective in treating various infectious diseases and reducing mortality rates for over 50 years. However, the evolution of antimicrobial-resistant strains of microorganisms poses a major challenge in managing infections. It seems that developing and using more powerful antibiotics has only short-term and partial benefits and may ultimately exacerbate the resistance problem (Huh & Kwon, 2011). As is presented in the Figure 1, shortly after the introduction of penicillin, bacteria developed resistance mechanisms, such as the production of the enzyme penicillinase which can inactivate the antibiotic (Huh & Kwon, 2011).

Furthermore, antibiotic misuse can lead to adverse outcomes, including the escalation of antibiotic resistance among bacterial populations and toxicity from high dosage administration. A fully new class of antibiotics has not been released in years since it is very challenging to bypass growing bacteria’s resistance mechanisms. The medicinal application of natural products has been recognized for centuries, and some exhibit inherent antibacterial properties. Historical evidence suggests that ancient Egyptians employed mold-infested bread in wound care practices to mitigate the risk of infection (Malczak & Gajda, 2023). Some natural compounds have either synergistic or additive effects when combined with antibiotics, indicating their prospective utility in antibacterial treatment options aimed at mitigating antibiotic toxicity and reducing the progression of bacterial resistance (Malczak & Gajda, 2023).

Antimicrobial resistance is not a problem just on the local level, but also on national, regional, and international, as no geographic boundaries can stop the resistance from

spreading (Manyi-Loh et al., 2018). In 2000, the World Health Organization (WHO) identified antimicrobial resistance as a significant global public issue, and by 2050 ten million resistance-related deaths per year are expected (Himanshu et al., 2023).

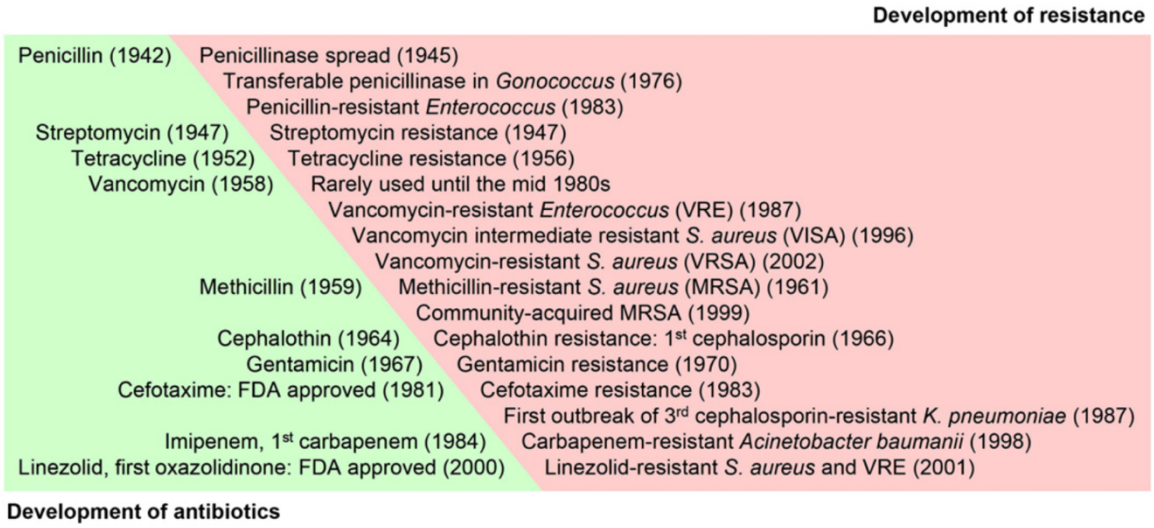

Figure 1: History of antibiotics and resistance development (Huh & Kwon, 2011).

1.1.2 Mechanisms of resistance

Bacteria are highly adaptive organisms and are able to overcome diverse threats in the environment, like toxic attacks and waste products coming from other organisms; among these mechanisms of defense, bacteria develop antimicrobial resistance (Blair et al., 2015). Resistance mechanisms vary among bacteria species; however, these mechanisms can counteract the vast majority of antimicrobials. The primary mechanisms of resistance involve limiting the uptake of a drug, altering drug targets, deactivating drugs, and active the efflux of a drug (Figure 2). These mechanisms can either be inherent from other microorganisms or acquired from other sources. A deeper understanding of these mechanisms holds promise for improving treatment options for infectious diseases and developing antimicrobial drugs that are more resilient to microbial resistance mechanisms (C Reygaert, 2018). Multidrug-resistant (MDR) bacteria are resistant to multiple classes of antibiotics, and it is particularly challenging to treat these with standard antibiotic therapies (Džidić et al., 2008). Bacteria may also present several distinct molecular

resistance mechanisms at once. MDR was identified in different bacterial species, such as methicillin-resistant *S. aureus* (MRSA) which examined a group of Rao et al. (2023), resulting in resistance to up to twelve different antibiotics. Secondly, extended-spectrum β-lactamases (ESBL) producing *E. coli* (Laconi et al., 2023), as they showed resistance to critically important antimicrobials other than third-generation cephalosporins. Ibrahim et al. (2023) highlighted the spread of ESBL-producing *E. coli* importance confirming MDR in most tested isolates (i.e., resistance to 4–16 out of 18 antibiotics tested). Both bacteria mentioned present a serious threat and can induce infections that are difficult to manage and are eventually life-threatening.

1.1.2.1 Mutations

Bacteria can acquire mutations in their DNA, which provide them with resistance to antibiotics, allowing them to survive in the presence of these drugs. Numerous genes can contribute to antibiotic resistance, either due to the presence of multiple targets, access points, or protective pathways for the antibiotic within the bacterial cell. Mutations can be spontaneous mutations or adaptive mutations. Mutational events can lead to the overproduction of antibiotic inactivating enzymes, or to activating the efflux systems (Džidić et al., 2008).

1.1.2.1.1 Horizontal gene transfer

Horizontal gene transfer is the acquisition of resistance genes from other microorganisms, and it is the main mechanism for the spread of antibiotic resistance. It occurs between different species, or even genera, including commensal-pathogens spread.

The main methods of horizontal gene transfer are conjugation, transformation, and transduction (Džidić et al., 2008). Conjugation is a typical resistance mechanism in Gram-negative bacteria, and mobile genetic elements (e.g. plasmids) are involved (Leungtongkam et al., 2018). Conjugation is the most significant and actively investigated type of horizontal gene transfer. Via transformation bacteria directly integrate free DNA fragments, and no contact (like in conjugation) is needed. Transduction is done due to phages (Liu et al., 2024).

1.1.2.2 Efflux pumps

Active efflux pumps transport drugs against their concentration gradient through the membrane (Kumar et al., 2013), therefore antimicrobial agents are expelled before they reach their target. While certain efflux pumps exhibit specificity towards specific substrates (e.g. Tet pumps), many demonstrate broad substrate specificity, enabling them to transport various chemically distinct compounds, earning them the designation of MDR efflux pumps (Blair et al., 2015). Evidence of the role of efflux pumps in biofilm resistance has been discovered in *Pseudomonas aeruginosa*, *E. coli*, and *Candida albicans* (Soto, 2013).

1.1.2.3 Altering drug targets

Antibiotics typically bind to specific targets with high affinity, thereby preventing these targets from functioning normally. Resistance to antibiotics occurs when the structure of the target is altered in such a way that antibiotics are unable to bind to it (Blair et al., 2015). This can be due to natural variations or acquired mutations in the target sites, like spontaneous mutation of a bacterial gene. This type of resistance is typical for pathogens such as methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecium*, *Streptococcus pneumoniae,* or *E. coli* (Kapoor et al., 2017).

1.1.2.4 Antibiotic inactivation

Some mechanisms involve the production of enzymes that can degrade or modify the target drug. Biochemical strategies encompass hydrolysis (e.g. hydrolytic amidases β-lactamases), group transfer (e.g. transferases), and redox mechanisms (Džidić et al., 2008). Extended-spectrum-lactamases (ESBL) producing *E. coli* are among the bacteria using this resistance mechanism (Laconi et al., 2023).

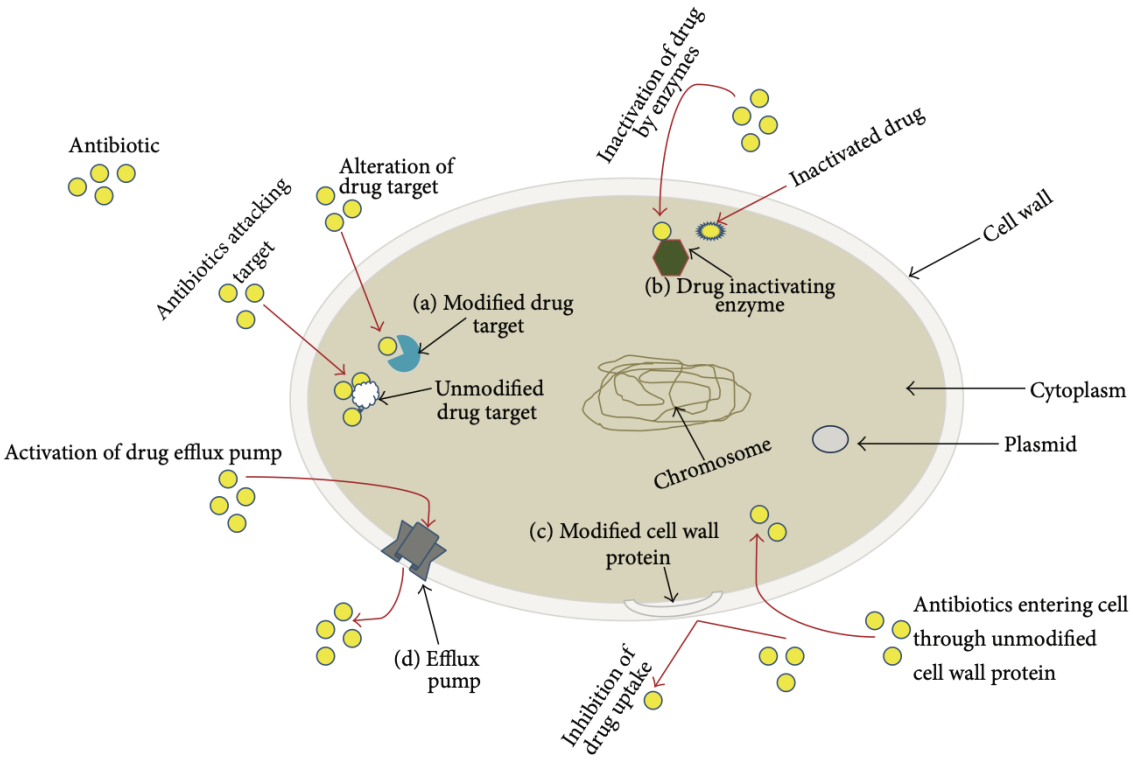

Figure 2: Summary of antimicrobial resistance mechanisms (Kumar et al., 2013).

1.1.2.5 Biofilm

Biofilm is a complex microbial association fixed to biotic and abiotic surfaces (Soto, 2013). In order to establish one, bacteria must collaborate on a series of related factors in the form of groups (Liu et al., 2024). It protects bacteria against mechanical, biological, and chemical agents (i.e., temperature, antibiotics, disinfectants), and its main goal is to adapt to a given ecological niche and effectively distribute the microbial population (Aleshukina et al., 2020). Resistant cells seem to form in deeper layers of thick and mature biofilms, and not in young colonies (Ito et al., 2009). Acquiring knowledge about biofilms plays an important role in managing various infections, as disruption of biofilms can significantly help with treatment (Aleshukina et al., 2020). Examples of bacteria known to form biofilm are *S. aureus*, *E. coli*, *P. aeruginosa*, *Enterococcus spp.*, *Salmonella spp.*, *Campylobacter spp.*, *Listeria spp.* (Aleshukina et al., 2020).

1.1.3 One Health

The use of antimicrobials is essential in maintaining the medical care and overall health of animals. However, it is crucial to recognize that the use of antimicrobials, whether in humans or animals, can contribute to the emergence and spread of antimicrobial resistance (European Commission, 2015). One Health is a field that connects humans, animals, and the environment, and it is presented in the Figure 3. This interdisciplinary field is fundamental as there is a mutual dependence of all three sections, and humans with animals also share risks for many infectious diseases. Bacteria found in animals can be transmitted to humans via the food chain and spread in the environment due to animal wastes. Mass medication of animals with antimicrobials that are critically important for humans (e.g. third-generation cephalosporins and fluoroquinolones), and the long-term use of medically important antimicrobials (e.g. colistin, tetracyclines, macrolides) for growth promotion are among the major concerns for animal health and agriculture sectors (McEwen & Collignon, 2018). Nevertheless, in the European Union, the prescription of antibiotics to animals must be justified by a veterinary diagnosis following the current status of scientific knowledge, and it is advisable to conduct antimicrobial susceptibility testing. Some antimicrobials are only authorized to be used in humans (European Commission, 2015). In the human sector, it is essential to prevent infections, reduce over-prescribing of antimicrobials, and improve sanitation as well as hygiene and infection control. Furthermore, the presence of resistance genes in an environment (i.e., resistome) is expanding. Numerous countries and international agencies have included a One Health approach within their action plans to address antimicrobial resistance. These actions include improvements in antimicrobial use regulation and policy, surveillance, stewardship, infection control, sanitation, animal husbandry, and alternatives to antimicrobials. World Health Organization (WHO) has launched new guidelines recommending that farmers and the food industry should stop using antimicrobials routinely to promote growth and prevent disease in healthy animals (McEwen & Collignon, 2018).

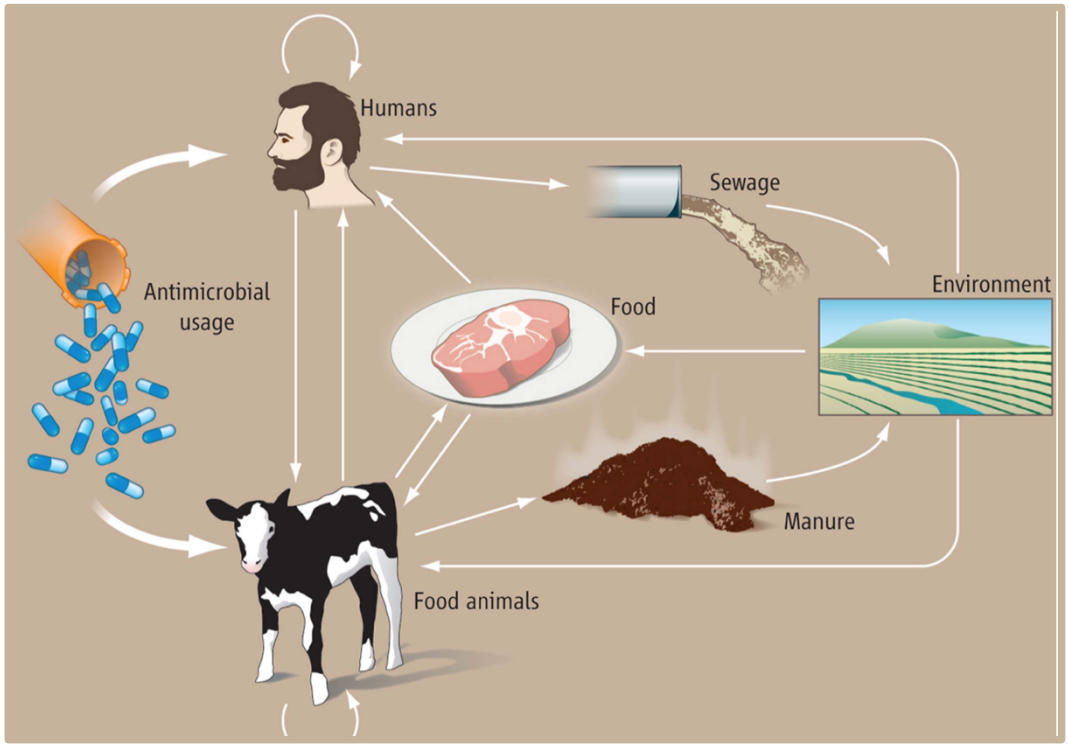

Figure 3: Interconnections of humans, animals, and environment (One Health) and antimicrobial resistance (McEwen & Collignon, 2018).

1.1.5 Antimicrobial resistance in livestock

The use of antibiotics in livestock is a contributing factor to the development of antimicrobial resistance (AMR) in both animals and humans (Christodoulou, 2023). Interacting and working with livestock present an increased risk of acquiring dangerous antibiotic-resistant strains (Christodoulou, 2023).

It is crucial to study how farmers employ antimicrobials and their knowledge and understanding of AMR. This could enhance their practices in antimicrobial usage and effectively address AMR issues in livestock production. Borelli et al. made a few conclusions from their survey, for example, that university degree holders are likely to have better knowledge about AMR, or that young and inexperienced farmers as well as larger farms reported higher antimicrobial use. Administering antimicrobials may seem like a quicker and simpler fix compared to the effort needed for monitoring and offering alternative forms of care (Borelli et al., 2023).

1.1.6 Important bacterial species

Some of the main pathogens occurring in livestock production are *Mannheimia haemolytica* causing respiratory infections in ruminants (mainly bovine and sheep) (Rice et al., 2008), *Staphylococcus aureus* and *Escherichia coli* resulting in bovine mastitis, or poultry infection (Sharma et al., 2023; Silva et al., 2014), *Streptococcus suis* which is one of the most important pathogens in pigs and its infection can result in septicaemia, meningitis, endocarditis, and pneumonia (Segura et al., 2020), *Pasteurella multocida* responsible for a wide range of diseases including bovine pasteurellosis (Dabo et al., 2008), and *Salmonella typhimurium* as an inducer of gastroenteritis and bacteraemia in animals (poultry, cattle, sheep) (Bawn et al., 2020).

1.1.6.1 Staphylococcus aureus

Staphylococcus aureus, a bacterium belonging to the *Staphylococcaceae* family, is an important zoonotic pathogen exhibiting significant wide-spread resistance to commonly used antibiotics, and thus representing a serious threat to both animal (bovine mastitis, poultry infection) and human health. Resistance in *S. aureus* occurs by either horizontal gene transfer or chromosomal mutations, and the mechanisms involved are enzymatic modification, efflux, target bypass, and drug displacement. Several phytochemicals show antibacterial effects against *S. aureus* (e.g. curcumin, gallic acid, eugenol, thymol) making them a successful alternative to commercial antibiotics (Sharma et al., 2023). Furthermore, *S. aureus* forms biofilm which increases the virulence and resistance in comparison to the planktonic state. In addition to that, in biofilms, most drugs present low permeability (Zhou et al., 2018). According to Zhou et al., 2018 nanoparticle drug delivery systems are a suitable weapon to combat these challenges.

Livestock can serve as reservoirs of methicillin-resistant *S. aureus* (MRSA); Rao et al., 2023 collected samples from small healthy-looking ruminants (sheep, goats) and bovine with overall positivity of *S. aureus* in 36% of samples and MRSA in 10% of samples. Some level of resistance was present against all tested antibiotics (twelve), covering many classes such as beta-lactams, tetracyclines, or macrolides. The tested strains were most prevalently resistant to penicillin and ampicillin, followed by tetracycline, methicillin, and kanamycin (Rao et al., 2023).

Chuprom et al., 2023 isolated staphylococci (*S. aureus* and *S. haemolyticus*) from bovine mastitis in dairy cows. Some isolates were resistant to the following antibiotics: penicillin, erythromycin, ampicillin, kanamycin, and trimethoprim. The researchers tested the antimicrobial activity of powdered wood extracts suggesting their successful use in livestock production. The extracts exhibited potential in growth inhibition, inhibition of biofilms, and adhesion of *S. aureus*, bacteria commonly associated with this inflammatory condition in cattle. This suggests that incorporating such extracts into treatment plans could serve as an effective strategy for managing bovine mastitis, potentially offering a natural and sustainable approach to address this concern (Chuprom et al., 2023).

1.1.6.2 Escherichia coli

Escherichia coli belongs to the *Enterobacteriaceae* family and can be found both as a commensal bacterium and as a pathogen causing various infections (Soto, 2013). In livestock production, *E. coli* is responsible for bovine mastitis which is an important disease in the dairy industry and milk production. In this case, the correct treatment needs to be chosen as well as sufficient concentrations of antimicrobials to avoid sub-inhibition (Silva et al., 2014).

Resistance mechanisms of *E. coli* present a serious threat to animal (poultry, bovine) and human health. Some *E. coli* are capable of producing extended-spectrum β-lactamases. These *E. coli* strains are referred to as "ESBLs.". Furthermore, some can produce plasmid-mediated AmpC β-lactamases. These genes located on mobile genetic elements can be transferred to non-resistant bacteria via a plasmid, and since *E. coli* also forms a biofilm, it makes a favorable environment for the exchange (Laconi et al., 2023). Moreover, ESBL-producing *E. coli* showed frequent multi-drug resistance (91% tested isolates) to various antibiotics including ampicillin, co-amoxiclav, cefotaxime, ceftazidime, ceftiofur, and cefquinome (Ibrahim et al., 2023). ESBL-producing *E. coli* emerges as a significant contributor to cephalosporin-resistant infections in both humans and animals (Ibrahim et al., 2023; Laconi et al., 2023).

Several metallic nanoparticles exhibit strong antibacterial action against *E. coli* (Sathiyaraj et al., 2021; Nithiyavathi et al., 2021), as well as polyphenolic extracts from various sources like black and green tea (Tiwari et al., 2005) or pine needle (Feng et al., 2010).

1.1.6.3 Streptococcus suis

Streptococcus suis is a commensal bacterium inhabiting the majority of pigs; however, in some, it causes serious disease. Naturally, *S. suis* resides in the tonsils and nasal cavities of pigs. Diseased pigs have lower performance and increased mortality, which have a significant economic impact on swine production worldwide. In animals, *S. suis* can cause septicaemia, meningitis, endocarditis, pneumonia, and arthritis (Segura et al., 2020). *S. suis* is classified within the "porcine respiratory disease complex," a term used to describe a common multifactorial respiratory disease in swine. This condition arises due to a combination of factors, including polymicrobial infections, environmental stressors, and the physiological status of the host animal (e.g. age and immune system strength) (Segura et al., 2020). *S. suis* is known to be highly resistant to tetracycline and erythromycin and less frequently also to florfenicol and penicillin. Carriage isolates obtained from asymptomatic individuals can cause lameness in livestock, although the survival rate is approximately 85%. On the other hand, higher mortality can be observed in pigs infected with the clinical isolates. Caesarean Derived Colostrum Deprived piglets are more likely to suffer from *S. suis* infection, as well as animals exposed to stressors (e.g. social stress, transport) (Segura et al., 2020).

Moreover, *S. suis* can infect humans in close contact with pigs or pork meat. The symptoms would be similar to those manifesting in animals: meningitis, sepsis, endocarditis, pneumonia, and arthritis (Segura et al., 2020). Biofilm formation is a common characteristic of *S. suis* and is considered to be an important virulence factor, and it can lead to persistent infection in both animals and humans (Y. Wang et al., 2018).

Qu et al., 2023 discussed the impact of polyphenolic compound gallic acid on restoring the sensitivity of multi-drug resistant (MDR) *S. suis*. They performed both, *in vitro* and *in vivo* experiments. The researchers found that the utilization of gallic can help to combat antimicrobial resistance by enhancing the effectiveness of sulphonamides against the MDR strain of S. suis. The gallic acid disturbed the integrity and function of the bacteria's cytoplasmatic membrane.

1.1.6.4 Mannheimia haemolytica

Mannheimia haemolytica is an opportunistic bacterium naturally colonizing the upper respiratory tract and nasopharynx of healthy ruminants. Nevertheless, if the host's immune system is compromised, *M. haemolytica* can access the lungs and result in respiratory disease

(Rice et al., 2008). *M. haemolytica* can be found in bovine often causing pneumonia in neonatal calves. In addition to that, infection from *M. haemolytica* can result in death due to pleuropneumonia (Rice et al., 2008). Rice et al., 2008 suggested that a combined strategy would be needed to tackle the spread of livestock production. Furthermore, *M. haemolytica* was found to be closely linked with cases of pneumonia also in sheep and goats (Girma et al., 2023). Girma et al., 2023 researched the extent of antibiotic resistance in *M. haemolytica*. Results showed some level of resistance in three out of six tested antibiotics. The susceptibility test revealed the greatest resistance to bacitracin and penicillin, with some resistance also noted for tetracycline. Furthermore, severe *M. haemolytica* infection in humans is unlikely, especially in infants and young children (Punpanich & Srijuntongsiri, 2012).

1.1.6.5 Pasteurella multocida

Pasteurella multocida is a zoonotic pathogen that can cause various diseases (J. Sun et al., 2024). It can be found in many animals, both in pets (cats, dogs) and in domestic animals (cattle, swine, poultry, rabbit). It colonizes the upper respiratory tract of animals, sometimes without any symptoms (Piorunek et al., 2023). In other cases, animals contracting *P. multocida* face severe, often fatal outcomes. This highlights the relevance of infections caused by this pathogen within animal populations, highlighting the significant threat it poses to their health and well-being (J. Sun et al., 2024). In livestock it can induce a respiratory disease (in some cases followed by the development of *P. multocida*-induced pneumonia) and septicaemia (Dabo et al., 2008). Humans can contract *P. multocida* through biting, scratching, and licking by infected animals. Indeed, it is the most common pathogen detected in wounds following animal bite or scratch (Piorunek et al., 2023). Inflammation emerges at the infection site and typically, it remains localized to the skin and subcutaneous tissue; however, under specific circumstances, it may extend to other organs, presenting as a severe systemic infection (Piorunek et al 2023). In humans, it may cause localized cellulitis, bacteremia, and septic shock (Lin et al., 2022). Lin et al., 2022 identified decreased sensitivity of *P. multocida* to certain antibiotics, including erythromycin (i.e., macrolide) (100%) and fluoroquinolones (37%), by modifying antibiotic target sites and regulating the flow of antibiotics into or out of bacterial cells, respectively.

1.1.6.6 Salmonella typhimurium

Salmonella typhimurium is an enteric pathogen known to be a leading cause of gastroenteritis and bacteraemia in humans and animals (Lamichhane et al., 2024). *S. typhimurium* infection is widespread among both domestic and wild animals, sometimes residing in their gastrointestinal tracts without manifesting evident symptoms of illness which contributes to the complex dynamics of *Salmonella* transmission (Demirbilek, 2018). Livestock (poultry, swine, cattle) can get infected by wild animals or by vectors (rats) (Lamichhane et al., 2024). *S. typhimurium* colonizing livestock can often affect human health and is commonly drug-resistant, whereas strains inhabiting wild animals (avian) are rarely associated with drug resistance and less frequently associated with human infection. This bacterium presents a risk to food safety and is considered to be a primary cause of foodborne illness (Bawn et al., 2020, Costabile et al., 2011). *S. typhimurium* can be transmitted to humans via contaminated food (Bawn et al., 2020). The main contributors are poultry and poultry products, followed by beef, pork, fish, and non-animal-derived food such as fruits and vegetables (Lamichhane et al., 2024).

Qin et al., 2022 detected frequent resistance to many antibiotics from various classes with the highest prevalence to tetracycline, ampicillin, sulfisoxazole, streptomycin (more than 75%), moreover also to nalidixic and amoxicillin-clavulanic acids (Qin et al., 2022). Antibiotics are pivotal for the treatment of salmonellosis; however, because of increasing antibiotic resistance and MDR strains (Lamichhane et al., 2024), an alternative strategy to antibiotics is necessary to control the spread of *Salmonella* infection in farm animals. Tannins, which could be used as feed additives, have been tested as an alternative (Costabile et al., 2011). Costabile et al. (2011) demonstrated the effectiveness of tannins against *S. typhimurium*. The team tested different sources of condensed and hydrolyzable tannins at different concentrations. Gallotannins were found to be particularly effective.

1.2 Nanomaterial

The term “nano” derives from the Latin “nanus”, which means dwarf or very small. Since 2005, this term has been increasingly applied in various scientific disciplines to refer to phenomena at the nanoscale. Nanoparticles (NPs) are particles of any shape with at least one dimension in the range from 1 to 100 nm. They refer to systems whose size exceeds the molecular scale but remains below the macroscopic scale. NPs can originate from both natural and artificial sources and are widely distributed in the environment as well as the laboratory settings. Nanoparticle’s properties change with their size, and many important and attractive properties express only at the nanoscale (Kafle, 2020). Additionally, through manipulation at the nano-scale, it is possible to modify the characteristics of materials, thereby attaining the desired properties essential for specific applications (Rezić, 2022). NPs tend to develop aggregates which together with other NP’s transformations can change the physiochemical properties, reactivity, transport, fate, and biological interactions (Zhang, 2014). Naturally occurring nanoparticles have been existing for millions of years. They have some characteristics that are appealing for biomedical use, like the uniformity of the structure, low toxicity, and aptitude to resist the defence system (Stanley, 2014). NPs exhibit high bioavailability and tissue-specific delivery. They persist longer in the cellular environment and bypass some of the clearance mechanisms that are normally effective, enabling them to cross the intestinal barrier. However, nanoparticles face the challenge of the immune system, which can recognize and eliminate them as foreign entities. To evade the immune response, nanoparticles form a protein layer (i.e., protein corona), which is represented in the Figure 4. Protein corona masks NPs’ surfaces and reduces their immunogenicity. It establishes a new biological identity that determines the outcome of nanoparticle administration. Consequently, it is fundamental to understand the impact of protein corona on the *in vivo* trajectory of nanoparticle delivery and explore strategies for exploiting it to enhance delivery efficiency (Y. F. Wang et al., 2023).

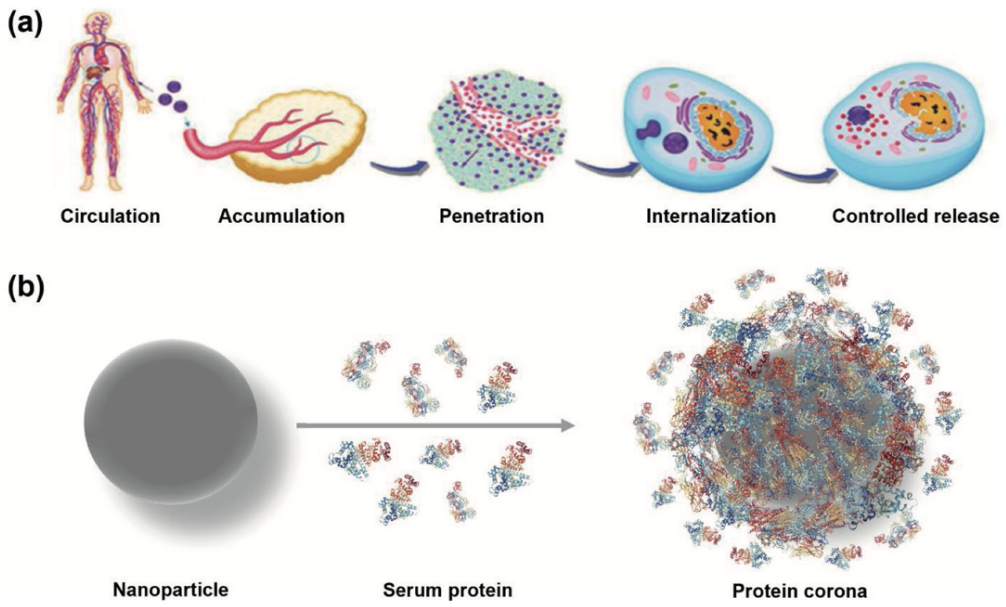

Figure 4: a) The delivery of NP in the human body; b) Protein corona and NP (Y. F. Wang et al., 2023).

1.2.1 Synthesis

There are numerous ways how to synthesize nanomaterials, but they are all categorized into two groups: top-down and bottom-up. Top-down means breaking down a bulk material into nano-sized objects (see Figure 5) by manufacturing or a natural process. This process is industrially used for making all current microelectronics. One example of this approach is making nanostructures by removing some crystal planes that are already on the substrate. A facet of top-down syntheses is the generation of imperfect surfaces displaying damages in the crystal structure (Kafle, 2020); however, this can lead to nanoparticles with unique properties. For instance, mechanical milling can produce nanomaterials with enhanced mechanical properties due to the fine dispersion of particles (Prasad Yadav et al., 2012). Top-down protocols include some strategies like chemical vapor deposition, milling process, and physical vapor deposition (Chinecherem Nkele & I. Ezema, 2021). This synthetic strategy usually gives better control, but it can only make a limited number of structures (Kafle, 2020). As an example, nanoparticles of iron oxide, carbon, and cobalt oxide have been produced using this method (Chinecherem Nkele & I. Ezema, 2021).

The bottom-up synthesis of nanoparticles involves the assembly of a material from the bottom. Atoms, molecules, or nanoparticles are used as the building blocks for the

fabrication of complex nanostructures. The size of the components depends on the properties that are desired. For example, the bottom-up fabrication of nanostructures can involve the deposition of atoms (or molecules) on a surface, resulting in a crystal plane formation, and then the stacking of more crystal planes leads to the formation of nanostructures.

The bottom-up approach is not a novel concept, nature provides many examples as all living organisms in nature grow by this approach (Kafle, 2020), as well as ancient manufacturing. The Lycurgus Cup is an extraordinary piece from the ancient glass industry (4th century) as well as an example of the nanoparticle's use, although unknowing (Freestone et al., 2008). The benefit of bottom-up is the cost (lower), scalability, and generally better uniformity of the product (Kafle, 2020). The employed methodologies typically involve processes such as green synthesis, biochemical methods, spin coating, and sol-gel techniques. The bottom-up approach has been utilized for the synthesis of nanoparticles composed of titanium dioxide, gold, and bismuth (Chinecherem Nkele & I. Ezema, 2021).

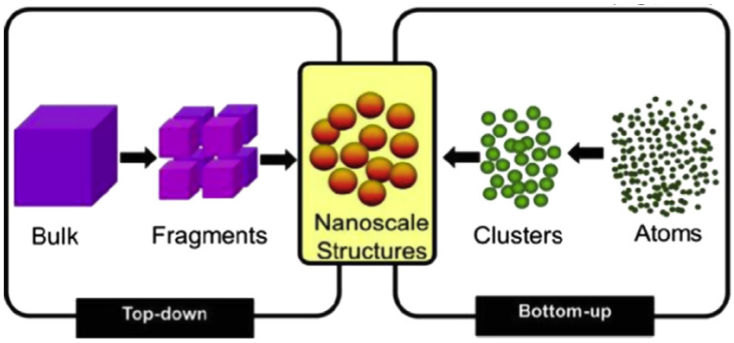

Figure 5: Top-down and bottom-up approaches (Kafle, 2020).

Another type of classification can be mentioned in three general techniques: physical, chemical, and biogenic methods (green synthesis). Examples of physical synthesis are bar milling, microwave-assisted, laser ablation, and sputtering. Physical processes are top-down syntheses, and they can produce pure nanoproducts, however costly instruments are needed. Examples of chemical methods are sol-gel or co-precipitation. Chemical methods utilize solely the bottom-up approach. Green synthesis uses plant and microbial extracts (bacteria, fungi, algae, yeasts). This synthetic strategy is eco-friendly, inexpensive, simple, and with minimal toxicity (Naz et al., 2023).

1.2.2 Applications

Nanotechnology finds application in modern agriculture, providing new efficient agrochemical agents, and sustainably improving crop productivity while reducing the need for potentially harmful pesticides (Singh Sekhon, 2014). In the food sector, nanotechnology has great potential in the development of functional foods, colorants, new flavours, and textures (Afroz et al., 2012; Singh Sekhon, 2014). Another field of nanoparticle application is biomedicine, in the development of cardiovascular implants and drug-delivery systems, for their antibiofilm activity, antitumor activity, wound healing properties, antifungal, antibacterial, antiviral and antiparasitic activity (Almatroudi, 2020). With the worldwide issue of growing antimicrobial resistance, nanoparticle's antibacterial action is particularly important as nanoparticles can be effectively used against multidrug-resistant pathogens. Different nanoparticles were studied against various bacteria. For example, silver nanoparticles (Zarei et al., 2014; Almatroudi, 2020; Hayat et al., 2023; Chapa González et al., 2023), gold nanoparticles (Su et al., 2020); L. Sun et al., 2023) and maghemite nanoparticles (de Almeida Roger et al., 2018) have been studied for their potential use as antimicrobial agents (medicines, cleaning products, against plant pathogens).

Furthermore, the physical and chemical interactions occurring between nanoparticles and pharmaceuticals are used in drug delivery. This facilitates controlled and targeted drug release to specific locations in the body, which favours their use as medicines (Wijaya et al., 2021).

To ensure the safety of the synthesized NPs, the optimization step should involve varying the key parameters that affect the size, shape, and composition of the NPs. By controlling these features, the potential of the NPs to generate reactive oxygen species (ROS) and induce cytotoxic, genotoxic, and neurotoxic effects can be reduced or eliminated (Rezić, 2022).

1.2.3 Nanoparticles and microorganisms

The use of antimicrobial NPs instead of antibiotics could have several advantages, including the above-mentioned targeted drug delivery and controlled drug release, low antimicrobial resistance, and broad therapeutic index. However, NPs exhibit slow elimination therefore there's a possibility of undesired accumulation in tissues and organs, and systemic exposure even to locally administrated drugs.

Mechanisms of antimicrobial NPs include reactive oxygen species (ROS) production, which leads to oxidized cellular components, DNA damage, and cell membrane disruption. Moreover, transmembrane electron transport is interrupted, and some heavy metal ions are released which can cause mitochondria and DNA damage.

Numerous NPs have been investigated as effective carriers for antibiotics, simultaneously acting as protective agents for antimicrobial drugs against resistance mechanisms in target microorganisms (Huh & Kwon, 2011).

1.2.4 Metallic nanoparticles

Numerous types of metal and metal oxide nanoparticles, including silver, silver oxide, gold, iron oxide, nickel, titanium dioxide, copper oxide, zinc oxide, calcium oxide, and magnesium oxide, have been identified for their antimicrobial properties. The distinctive features inherent to metallic nanoparticles have been effectively utilized in both diagnostic and therapeutic applications (Hante et al., 2019).

1.2.4.1 Silver nanoparticles

Silver nanoparticles (AgNPs) have shown extensive antibacterial activity against many bacteria commonly causing infections (*E. coli*, *Bacillus subtilis*, *S. typhimurium*, *S. aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Listeria innocua* and *Listeria monocytogenes*) (Hayat et al., 2023; Zarei et al., 2014). AgNP's antimicrobial attributes have been extensively documented which resulted in their pervasive application in both commercial and biomedical contexts. Consequently, silver nanoparticles have attained preeminence as the most widely commercialized nanoparticles (Hante et al., 2019). The bacterial outer cell membrane has an important role in the interaction with AgNPs, leading to its integrity disruption, resulting in the release of intracellular contents and cell death. Additionally, AgNPs have shown to be more effective against Gram-negative bacteria and that is because of their membrane composition which is highlighted in the Figure 6. Gram-positive bacteria have a thick and exposed peptidoglycan layer, which differs in the structure of the polysaccharide chains from that of Gram-negative bacteria. Moreover, AgNPs preferably bind to the membrane of Gram-negative bacteria (Chapa González et al., 2023). AgNPs can be also used in combination with other antibacterials. Two-sided antibacterial cellulose combining probiotics and AgNPs was effectively used to inhibit an opportunistic pathogen *P. aeruginosa*. A combination of antibacterials in a biocompatible biomaterial could become a promising strategy for treating infections caused by a broader spectrum of resistant pathogens (Sabio et al., 2021). In addition to that, AgNPs' action can be enhanced by synergizing with another metallic nanoparticle. Vasiliev et al., 2023 confirmed the positive synergic effect between AgNP and another nanoparticle (copper oxide NP), the antibacterial effects of AgNP were enhanced up to six times. The suggested use could be as a wound care product, to cure topical bacterial infections.

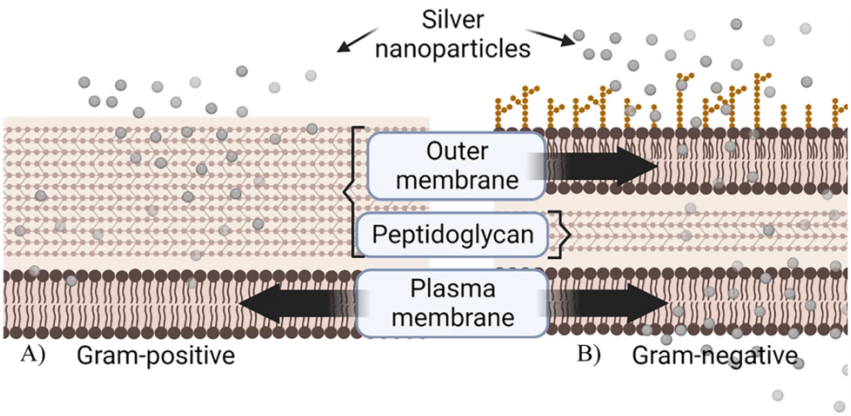

Figure 6: AgNPs interaction with Gram-negative and Gram-positive bacteria (Chapa González et al., 2023).

1.2.4.2 Gold nanoparticles

Gold nanoparticles (AuNPs) exhibit excellent stability, and biocompatibility and can also act as effective drug carriers. AuNPs can synergize with antibacterial agents (antibiotics, cations, low-temperature plasma) to overcome the resistance of some bacteria. Moreover, AuNPs also have photothermal effects, which can be exploited for photothermal therapy to eradicate bacteria (Su et al., 2020). AuNPs can directly target cellular biological processes to influence cell fate and function. Other than their antibacterial action, they are widely explored for cancer diagnosis and therapy. Recent evidence suggests that AuNPs can act as antitumor agents by modulating cellular processes, but the underlying mechanisms are not fully understood (L. Sun et al., 2023).

AuNPs are harmful to various bacterial strains, their inhibitory action has been proven for example against *Klebsiella pneumoniae*, *E. coli,* and *B. subtilis*. Metal ions in solution disperse around the bacterial cell wall, and some NPs engage with the cell wall which leads to an increase of cell toxicity. Again, AuNPs are more effective against gram-negative bacteria (thinner cell wall) (Sathiyaraj et al., 2021).

However, in both cases (silver and gold nanoparticles), it is necessary to conduct more research regarding their toxicity, especially after a long time and large dose. If they reach the bloodstream, potentially they could interact with the blood components and eventually induce bleeding or thrombosis (Hante et al., 2019).

1.2.4.3 Copper oxide nanoparticles

Copper oxide nanoparticles (CuO NPs) are an example of metal oxide nanoparticles with good antibacterial activity. CuO NPs work on both, Gram-negative and Gram-positive bacteria, and are widely used in hospitals as antibacterial coating on various types of medical equipment. For example, they were proven to inhibit Gram-positive *S. aureus*, then Gram-negative *E. coli*, *K. pneumonia*, *S. typhimurium,* and yeast bacteria *Candida albicans* (Nithiyavathi et al., 2021). In addition, CuO NPs have shown many important properties in different areas of biomedicine, like antioxidant, anticancer, antiparasitic, antidiabetic, and antiviral activities. The toxic effects of CuO NPs are mediated by the generation of reactive oxygen species (ROS), which trigger a series of biochemical events. These include the alteration of enzymatic activities, the peroxidation of lipids, the denaturation of proteins and nucleic acids, the damage of organ structures and functions, and the impairment of hematological and metabolic parameters. In general, toxicity and biological function are determined by the dose and concentration of NPs (Naz et al., 2023).

1.2.4.4 Iron oxide nanoparticles

Iron oxide nanoparticles (IONPs) are composed of magnetite (Fe_3O_4) or maghemite (Fe_2O_3, γ) (Boyer et al., 2010) and have demonstrated antimicrobial effects against a broad spectrum of microorganisms (bacteria, fungi, parasites, viruses) as well as synergic effect with traditional antibiotics (Zúñiga-Miranda et al., 2023). Due to their outstanding nanoscale physical properties, such as magnetism/superparamagnetism, IONPs have found numerous applications in various critical domains. In biomedical contexts, they are typically considered biologically inert. Nevertheless, IONPs also exhibit inherent enzyme-like activities, and thus they are recognized as new enzyme mimetics. The term "Nanozyme" has been introduced to emphasize the intrinsic enzymatic characteristics of these nanomaterials. Their behaviour mimics those of peroxidase and catalase, therefore the activities are similar to natural enzymes (Gao et al., 2017). In medicine, IONPs serve as contrast agents for Magnetic Resonance Imaging (MRI) and as elements in hyperthermia therapy, and they can be used in drug or gene delivery. IONPs are not stable in water/physiological fluids, however, there can be many different modifications to their surface to increase their stability (e.g. small organic compounds or polymer chains coating) (Boyer et al., 2010).

1.2.4.5 Maghemite nanoparticles – SAMNs (γ-Fe_2O_3)

A novel class of magnetic nanoparticles (surface active maghemite nanoparticles; SAMNs) is composed of nanostructured superparamagnetic iron oxide with stoichiometric maghemite (γ-Fe2O3) having an average size of 10 nm (Magro, Faralli, et al., 2012; Magro, Sinigaglia, et al., 2012). The final synthesis product is a brown nanopowder which shows a magnetic response when exposed to a magnetic field (Magro, Faralli, et al., 2012). In the form of colloidal suspension, surface active maghemite nanoparticles display peculiar surface chemical behaviour, remarkable water stability, and a high magnetic moment enabling easy magnetic manipulation. OH^- groups present on their surface stabilize the nanoparticles. These OH^- groups act as electrostatic barriers to prevent aggregation, and they facilitate reversible binding of various organic substances with opposite charges. Organic molecule and SAMN surface mutual interaction results in nanoparticle optical properties modification (Magro, Sinigaglia, et al., 2012). Due to these characteristics, SAMNs have been already used and tested for different purposes.

Another study combines maghemite nanoparticles (SAMNs) with polyphenolic compounds and investigates their interaction with bacteria. Indeed, the team of scientists de Almeida Roger et al., 2018 discovered that tannic acid nanocarrier (SAMN@TA) can be used to inhibit the growth of *L. monocytogenes* and can be considered a cost-effective and environmentally sustainable option for cleaning purposes and to decrease the growth and level of contamination in food processing (de Almeida Roger et al., 2018).

1.2.4.6 Hematite nanoparticles (α-Fe_2O_3)

Hematite nanoparticles have garnered significant attention due to their stability in air under ambient conditions as well as for their wide broad applications in various fields. They are not superparamagnetic but are known to be weak ferromagnetic (Rufus et al., 2016). Sihem et al. (2020) discovered that α-Fe_2O_3@Ag combined with Urtica leaf extract has excellent antibacterial activity against both gram-positive (*S. aureus*, *Bacillus*) and gram-negative bacteria (*K. pneumonia*, *E. coli*), and higher compared to α-Fe_2O_3 on its own (probably due to synergic effect between silver and iron ions). In this case, the mechanism of antibacterial activity against selected bacteria was direct physical interaction with the bacteria leading to disruption of the cell membrane and induction of oxidative stress (Rufus et al., 2016; Sihem et al., 2020)

1.3 Phenolic compounds

Phenolic compounds are a large class of secondary metabolites in the plant kingdom. Phenolic compounds can be found in a large variety of natural products, such as fruits, vegetables, seeds, trees, and herbs (Waqas et al., 2023). Other than in wild and cultivated plants, phenolic compounds can be also found in agro-industrial product derivatives (i.e., products obtained from agricultural and industrial processes) and wastes (i.e., by-products from vegetable and fruit processing) (Nurzyńska-Wierdak, 2023). The main classes of polyphenols include flavonoids and non-flavonoids (tannins), and the classification is presented in the Figure 7 (Waqas et al., 2023). Their molecular structure consists of at least one aromatic ring which is substituted with one or more hydroxyl moieties, and most of them include in their structure at least two hydroxyls. The potential of phenolic compounds as antioxidants is determined by the quantity and arrangement of hydroxyl groups (Farhat et al., 2020). Phenolic compounds stand out as exceptional natural compounds, exhibiting a spectrum of health-promoting benefits. They have excellent biological activity: antioxidant, anti-inflammatory, antimicrobial, antiatherosclerotic, antidiabetic, antiallergic, prebiotic, and antimutagenic (Nurzyńska-Wierdak, 2023)

According to many research groups and studies (Costabile et al., 2011; Johny et al., 2010; Brus et al., 2018; Sivasankar et al., 2020) polyphenolic phytochemicals are a great candidate to be used as feed additives in livestock production to address and control antibiotic resistance issue, and additionally, they can improve overall animal health, enhancing growth, intestinal health, and feed efficiency (Al-Mnaser et al., 2022).

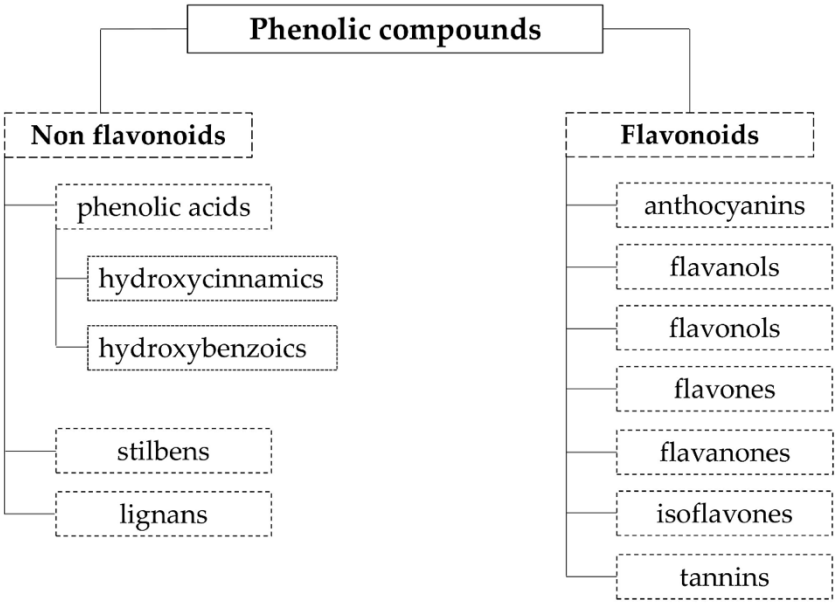

Figure 7: The main classification of phenolic compounds (Ferreira-Santos et al., 2020).

1.3.1 Tannins

Tannins constitute a diverse category of polyphenolic compounds with a wide range of molecular weights, distributed across various plant families, particularly prevalent in cereals, fruits, and vegetables (Sallam et al., 2021), as well as in wood, bark of trees, and herbs. The primary classes of these bioactive compounds include hydrolyzable tannins (e.g. chestnut wood), condensed tannins (e.g. wood, bark, grape skins) (Pizzi, 2021), and phlorotannins derived from marine algae. Examples of tannins are catechin, epicatechin, or epigallocatechin. Their bioavailability varies among different sites of the gastrointestinal tract; however, the microbiota-mediated hydrolysis of tannins gives rise to metabolites that are highly bioaccessible, and responsible for the majority of the health benefits associated with tannins consumption. Tannins exhibit numerous advantageous pharmacological effects, encompassing anti-inflammatory, antioxidant, antidiabetic, anticancer, and cardioprotective properties (Sallam et al., 2021). Moreover, they have been proven to have antibacterial activity (e.g. tannic acid, castalin, gallotannins, ellagitannins) against many bacteria like *S. aureus* (Štumpf et al., 2023), *E. coli*, *Shigella boydii,* and *Shigella flexneri*, *P. aeruginosa* (Modi et al., 2023), *S. typhimurium* (Costabile et al., 2011).

Tannins exert this action by forming irreversible bonds with proteins, a process that results in the formation of complexes within bacterial membranes. This interaction ultimately leads

to the neutralization of bacterial activity, making tannins valuable in combating microbial growth and infections (Pizzi, 2021).

As an example, drug-resistant *Salmonella* typhi and paratyphi A showed susceptibility to different phytochemicals and in particular to tannic acid. The tannic acid treatment resulted in the partial restoration of previously ineffective antibiotics due to resistance (ampicillin, ciprofloxacin, azithromycin). Moreover, tannic acid has exhibited anti-quorum sensing activity by inhibiting swarming motility, as well as anti-adhesion activity which subsequently prevents biofilm formation (Sivasankar et al., 2020).

On top of that, tannins demonstrated great potential to be utilized as feed additives to address infections in livestock production (Costabile et al., 2011). Animal breeders and producers of healthy food highly value the use of plant extracts that have high antioxidant potential, along with selective antimicrobial activity (Brus et al., 2018). Brus et al. (2018) tested powdered hydrolyzable tannins from chestnut as a supplementation in boiler production.

1.3.2 Polyphenol's antimicrobial activity

Numerous polyphenols (e.g. flavonols, tannis) found in different foods and beverages have exhibited antibacterial, antiviral, and antifungal activity. In the past years, this antimicrobial activity has been largely studied and a great number of publications have been released. Closely has been studied for example green and black tea, propolis, and berry extract due to their high concentration of polyphenols showing effectivity against several human pathogens (Coppo & Marchese, 2014). The strong ability of polyphenols to combat microbes largely arises from the presence of phenolic hydroxyl groups (Liu et al., 2024). Polyphenols can combat resistant bacteria directly, enhance the effectiveness of existing antibiotics through synergy, or reverse bacterial resistance to antibiotics (Turuvekere Vittala Murthy et al., 2021).

One way how polyphenols inhibit or kill microorganisms is by interfering with the physiology of bacteria which can be done by different mechanisms like disrupting the membrane functions or suppressing some virulence factors (enzymes, toxins, signal receptors, formation of biofilm) (Coppo & Marchese, 2014). Black and green tea effectively inhibit *S. typhimurium*, *Shigella dysenteriae*, *Yersinia enterocolitica,* and *E. coli* (Tiwari et al., 2005).

Moreover, polyphenols can create a synergic effect when combined with antibiotics. They modulate resistance in multi-resistant strains of *S. aureus* and ESBL-producing *E. coli*

(Coppo & Marchese, 2014). Polyphenolic compounds are capable of modulating and reversing resistance to several groups of antibiotics. They can reverse the resistance to β-lactam antibiotics (benzylpenicillin, oxacillin, methicillin, ampicillin, and cephalexin) in different bacterial species, for example, methicillin-susceptible and methicillin-resistant *S. aureus* (Stapleton et al., 2004; W. H. Zhao et al., 2001). The concentration of polyphenolic compounds (catechins) that Zhao et al. used was as little as around 10 μg/mL, whereas the concentration in beverages is usually 2 – 3 mg/mL (W. H. Zhao et al., 2001).

Tiwari et al. (2005) reported synergistic activity of flavonoids with chloramphenicol and other antibiotics like gentamycin (aminoglycosides), methicillin (beta-lactam), and nalidixic acid (fluoroquinolones) against diverse enteropathogens (*S. typhimurium*, *S. dysenteriae*, *Y. enterocolitica,* and *E. coli*).

Betts et al. (2019) concluded the ability of epigallocatechin gallate (EGCG) to restore the efficacy of β-lactam antibiotic aztreonam against multidrug-resistant strain of *P. aeruginosa* due to their existing synergy.

Furthermore, flavonol rutin has been proven to reduce biofilm formation (by reduction of cell viability or by lowering the amounts of exopolysaccharide production within the biofilm), and it exhibited several antimicrobial activities like interfering with membrane permeability or suppressing bacteria's virulence factors production. Tested bacteria were multidrug-resistant *P. aeruginosa*, methicillin-resistant *S. aureus*, *E. coli,* and others (Ivanov et al., 2022).

In addition to that, polyphenolic compounds could be used as a combined therapy and as a result lower the needed dosage of antibiotics which would avoid antibiotic side effects. Combined therapy can also improve antibiotic's pharmacokinetics and pharmacodynamics properties (Miklasińska-Majdanik et al., 2018).

1.3.3 Nanoformulations of polyphenols with antibacterial activity

Even though polyphenols demonstrate outstanding antibacterial potential, most of them present poor water solubility, short half-lives, and low bioavailability. Furthermore, environmental factors such as acidity, temperature variations, and enzymes can compromise the stability of these substances, thereby further limiting their therapeutic activity. Nanoformulations can overcome the existing shortcomings of polyphenols. It results in improved bioavailability and controlled drug release, which makes them more suitable for the biomedical field as well as livestock production (Liu et al., 2024). Moreover, it acts as

a protective shield for polyphenols, preventing their degradation, enhancing their absorption, and minimizing potential toxicity (Turuvekere Vittala Murthy et al., 2021).

Examples of existing nanoformulations that have been studied to improve characteristics of polyphenolic agents are polymeric nanoparticles, liposomes, hydrogels, microspheres, transferosomes, solid lipid nanoparticles, metal-nanoparticles, drug-polymer conjugates, and nanostructured lipid carriers (Turuvekere Vittala Murthy et al., 2021).

Several research groups have tested different types of nanoformulations of polyphenols to improve their characteristics. Dian et al., 2014 wanted to enhance the aqueous solubility and stability of polyphenolic compound quercetin using nanosized polymeric micelles. The outcomes were positive and other than the abovementioned, the compound's absorption was improved, and the half-life was extended (Dian et al., 2014).

Shababdoust et al. (2020) used curcumin-loaded nanofibers. As a result of this re-formulation, the compound's antibacterial activity against selected bacteria (*S. aureus* and *E. coli*) was significantly enhanced. In addition to that, the controlled hydrophilicity (how the surface interacts with water molecules) was improved as well (Shababdoust et al., 2020).

Huang et al. (2017) developed an innovative polymeric micelle for the simultaneous decorating of silver nanoparticles and encapsulating of curcumin (PM-Ag-Cur), aiming to enhance antibacterial effectiveness (against *P. aureginosa* and *S. aureus*) through a combined approach. The results confirmed this hypothesis, and the authors suggested that polymeric micelles could serve as a sufficient nanocarrier to overcome certain problems, like low aqueous solubility and bioavailability of curcumin (Huang et al., 2017).

De Almeida Roger et al. (2018) produced a study about the use of magnetically removable tannic acid nanocarrier SAMN@TA. They stated that the use of SAMN@TA as an antibacterial agent in the food industry would be both efficient and affordable, while also being an environmentally sustainable solution.

However, despite the interesting and promising outcomes of the studies mentioned, the use of nanoformulations highlighted may not be feasible for bacterial management in livestock production as the costs to fabricate such nanoparticles could be too high.

1.3.4 Wood industry and polyphenols

Every year a significant amount of wood waste is created. If this wood waste is dealt with improperly (e.g. burning it), it will eventually lead to increased greenhouse gases in the

atmosphere. Instead, it can be utilized as a component of new and innovative products (Maier, 2023).

Wood waste derived from forest trees contains various bioactive compounds that have potential applications in the food and pharmaceutical sectors (Smailagić et al., 2020). The main bioactive compounds are the following; polyphenols (e.g. flavonoids and non-flavonoids), stilbenes (e.g. resveratrol), terpenes, and alkaloids (Aliaño-González et al., 2022).

Extracting and purifying these bioactive molecules or extracts from plant materials is crucial for utilizing natural resources effectively in subsequent industrial processes (Ferreira-Santos et al., 2020). Various wood waste extracts show antimicrobial activity, for example against *Streptococcus mutans, Streptococcus pyogenes*, *L. monocytogenes*, *S. aureus*, *E. faecalis* and *E. coli* (Smailagić et al., 2020).

In Europe, pine forests stand out as one of the most widespread types of forest formations. Consequently, pine residues and by-products emerge as significant reservoirs of compounds holding considerable industrial value, and they also play a crucial role in the production of bioenergy. Pine extracts draw a lot of attention due to their favorable bioproperties, including anti-inflammatory, antimicrobial, anti-neurodegenerative, antitumoral, and cardioprotective effects, among others. These extracts hold promise for diverse industrial applications, serving as functional foods, preservatives in food additives, nutraceuticals, pharmaceuticals, and cosmetic ingredients. The bioactivities are largely attributed to the presence of phenolic compounds (Ferreira-Santos et al., 2020).

Utilization of pine barks leads to increased use of agroforestry by-products and residues, thus bringing the waste back to the market. Pine contains diverse polyphenol compounds, for example, p-coumaric acid and epicatechin (needles), eriodictyol and taxifolin (seeds), catechin, gallic acid, and taxifolin (bark) (Ferreira-Santos et al., 2020).

Feng et al. (2010) suggested that pine needles could be utilized as a safe and efficient antibacterial agent. They successfully tested its inhibitory action against diverse pathogenic bacterial strains, like *Bacillus subtilis*, *S. aureus*, *Bacillus cereus*, or *E. coli*.

2 Materials and methods

2.1 Materials

The chemicals, which had the highest purity acquirable, were used without any additional treatment. The medium Blood Agar (BA), Muller Hinton Agar (MHA), Brain Heart Infusion (BHI) broth were obtained from Aldrich (Sigma–Aldrich, Italy). Petri dishes, Eppendorf tubes, falcon tubes, tips, inoculation loops, and 96 microtiter plates for Minimum Inhibitory Concentration (MIC) were single used. Sterile water was used during the process.

The origin of the polyphenolic nanoparticles is the wood industry, and their suspension was provided by AINT s.r.l. (Advanced Iron Nano Technologies, Venice, Italy).

2.1.1 Medium/broth

A culture medium has to contain all the essential nutrients, ions, and moisture, to be able to maintain the correct pH and osmotic pressure and to neutralize any toxic materials produced (Greenwood D, S. R., 2006). To some extent, the used medium should simulate the environmental conditions of the pathogens' natural habitat. Host-mimicking medium contains host-derived factors, carbon, nitrogen, and other components relevant to a specific infection or found within a specific tissue (Belanger & Hancock, 2021).

Blood Agar (BA) is a type of solid culture medium that contains blood as a source of nutrients and hemin for the growth of bacteria. In developed countries, blood agar prepared with defibrinated sheep or horse blood is a standard way how to isolate different bacteria from clinical samples. It is useful for the isolation and identification of bacteria based on their haemolytic properties (Niederstebruch et al., 2017).

Muller Hinton Agar (MHA) is a non-selective and non-differential medium, and it allows the growth of most bacteria. MHA is also widely used for antimicrobial susceptibility testing. Starch in the medium absorbs toxins liberated from bacteria and minimizes the inhibitory action of antibiotics. Antibiotics can diffuse better in MHA compared to other agars (Salam et al., 2023).

Brain heart infusion (BHI) is composed of calf brain, beef heart, peptone, dextrose, sodium chloride, and disodium phosphate. BHI is a versatile nutrient medium that can be used to

grow different kinds of microorganisms such as bacteria, yeasts, and mold (Yang et al., 2018).

2.1.2 Bacteria

The bacterial species chosen for this experiment are of great interest for their relevance in livestock production, animal and human health, and potential antimicrobial resistance. The bacterial identification and purity were confirmed at the genus and species level before performing the Minimum Inhibitory Concentration (MIC) assays. The inoculum concentration of bacteria was standardized and consistent for the accurate measurement of the antimicrobial activity. The protocol described below is applicable to other strains and isolates. The bacterial strains used in this experiment are listed along with their respective strain IDs, initial concentration, and other important information in the Table 1.

Table 1: Characteristics of used bacteria

Bacteria	Strain ID	log cfu/ml	Solid medium	Liquid medium
Pasteurella multocida	5029/4	7,944	Blood agar	BHI
Stafilococcus aureus	NA	8,086	Blood agar	BHI
Streptococcus suis	5001	7,591	Blood agar	BHI
Mannheimia haemolitica	5029/3	5,881	Blood agar	BHI
Escherichia coli **(APEC)**	F2A2	11,057	Blood agar	BHI
Salmonella typhimurium	NA	7,763	MHA	BHI

2.2 Instrumentation

Optical spectroscopy within the ultraviolet and visible light range (UV/VIS) finds extensive application across diverse market segments and professional environments, encompassing research, production, and quality control. UV/VIS spectroscopy (Figure 8) is based on the absorption of light by a sample. The sample is illuminated with electromagnetic rays of various wavelengths in the visible (VIS) and ultraviolet (UV) ranges and part of the lower infrared (IR) region. Each substance absorbs light differently, thus unique relationships occur between the substance and its UV/VIS spectrum. Utilizing the correlation between

absorbance and sample concentration, it serves as a quantitative analytical method within various market sectors, including Water Testing, Food and Beverages, Pharmaceutical, Chemical, and Biotechnology industries. In qualitative analysis, UV/VIS spectroscopy can be used to ascertain the purity and absence of decomposition in the analyte. This methodology is particularly applied in the quality control assessment of incoming raw materials, as well as in the purity validation of biologically significant compounds (DNA, RNA) (De Caro, Cosimo; Haller, 2015) In microbiology, spectroscopy methods can analyze and characterize in real-time microbial populations. Spectral measurements can provide quantitative information on how microorganisms grow by capturing the changes in their spectra over time (Alupoaei & García-Rubio, 2004). UV/VIS spectroscopy can quickly detect the presence of microorganisms in a sample, helping to prevent foodborne diseases caused by foodborne pathogenic bacteria (X. Zhao et al., 2018).

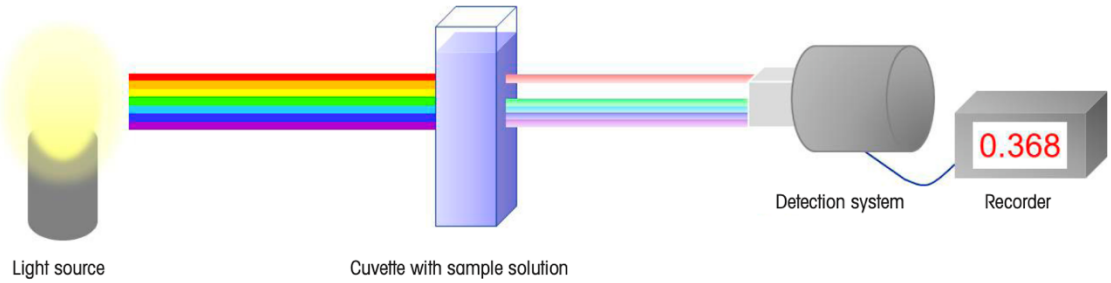

Figure 8: Measurement principle in UV/VIS spectroscopy (De Caro, Cosimo; Haller, 2015).

2.3 Minimal Inhibitory Concentration

The Minimal Inhibitory Concentration (MIC) assay uses either agar or broth dilution methodologies to assess, within specified experimental parameters, the minimum effective concentration of an antimicrobial agent, which inhibits the observable growth of a specific target bacterium. This assay serves the purpose of evaluating the susceptibilities of bacterial isolates as well as newly developed antimicrobial drugs. It is commonly conducted in nutrient-rich laboratory media that have limited relevance to *in vivo* conditions (Belanger & Hancock, 2021). The microdilution method miniaturizes the dilution method by using microliter volumes, this way multiple antimicrobials and/or bacteria can be tested simultaneously on a single microtiter plate (Puttaswamy et al., 2018; Salam et al., 2023).

2.4 Experimental protocol

The experimental part of the study aimed to determine the Minimum Inhibitory Concentrations of nanoparticle dilutions against a panel of six selected bacterial strains. All microbiological practices were carried out following all necessary safety rules and working under a microbiological safety cabinet which provides a safe working environment for researchers handling material potentially hazardous.

2.4.1 Bacteria resuscitation on Blood Agar (BA)

Frozen bacterial cultures were thawed in ice. Using the four quadrants technique, the bacteria were streaked onto sterile Petri dishes with BA/MHA medium in order to gain individual colonies. Each experiment was performed in duplicate. The plates were incubated at 37° C for 24 hours.

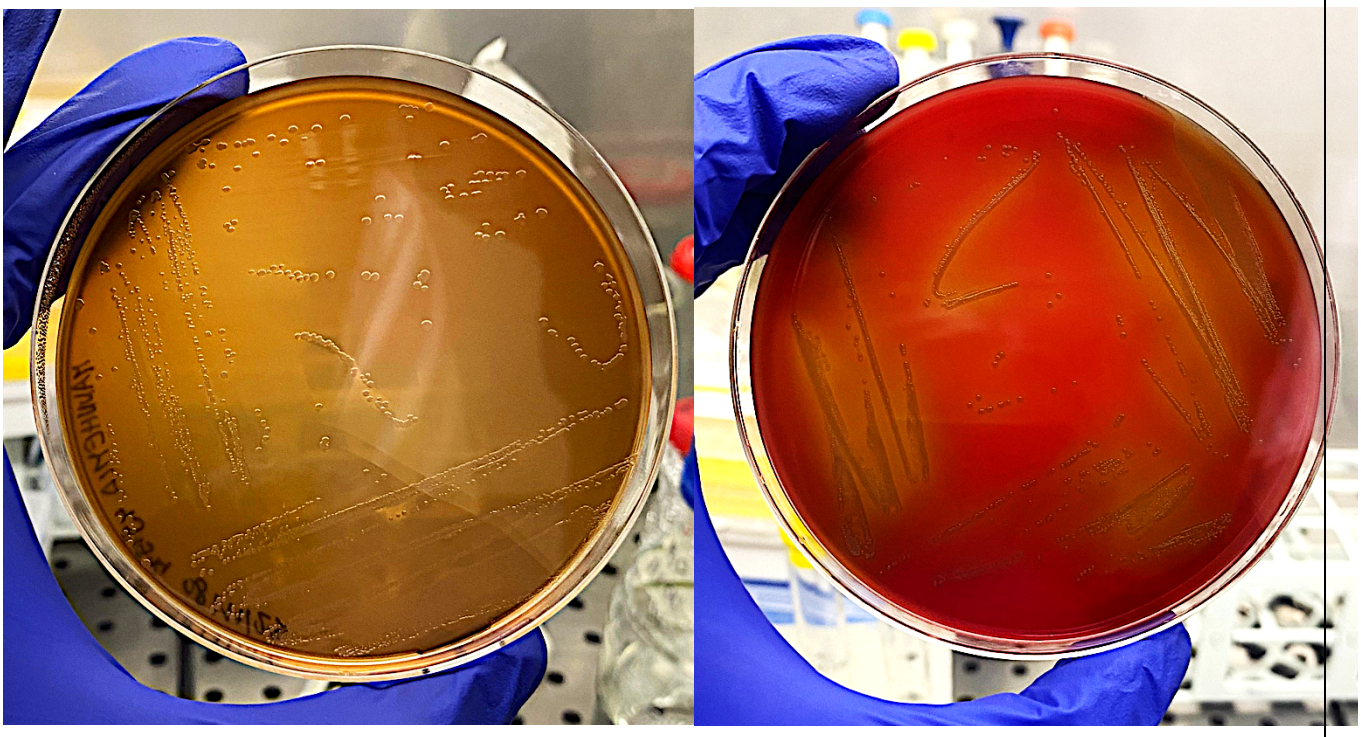

Figure 9: 9A *Mannheimia haemolitica*, 9B *Streptococcus suis*

2.4.2 Liquid culture

A single colony was isolated using a single-use inoculation loop and inoculated into 5mL of BHI medium and incubated at 37° C for 24 hours on a shaker. After 24 hours of incubation, plates were secured with parafilm and transported to the fridge.

2.4.3 Preparation of nanoparticle dilutions

The nanoparticle suspension was taken out of the fridge and placed under the cabinet, covered with aluminum foil to ensure maximal darkness. The initial concentration of the nanoparticle solution was 50 mg/L. After homogenization of the nanoparticle solution, the first dilution with a concentration of 15 mg/L was created by diluting the suspended nanoparticles 3,3 times. Using a pipette 7 mL of sterile water and 3mL of suspended nanoparticles were transported into a falcon tube and used to prepare five two-fold dilutions from 1/2 to 1/32. The final concentrations of each dilution are listed in Figure 3. Two mL Eppendorf tubes were used, together with sterile single-use tips and pipette. Subsequently, 750 μL of sterile water and 750 μL of correct dilution were added to each Eppendorf. All dilutions were vortexed before use, to ensure they were properly mixed and ready to use for our experiment. Closed Eppendorf tubes were secured with parafilm, placed in the fridge, and covered with aluminum foil to ensure maximal darkness.

2.4.4 Bacteria dilution

To range of bacterial concentrations, a systematic series of ten-fold dilutions was prepared. The desired final concentration was 5 log CFU/mL. As shown in the Table 1 each bacterial sample had a different initial concentration. Tubes contained 9 mL of BHI and 1 mL of inoculum, starting from the original bacterial suspension. The number of tubes prepared depended on the specific bacteria and its initial count.

2.4.5 Preparation of the microtiter plate

Sterile water, BHI broth, nanoparticle dilutions, and diluted bacteria (5 log CFU/mL) were used. The typical layout of the 96-well microtiter plate is illustrated in the Figures 10 and 11. Nanoparticle dilutions with a concentration ranging from 15 mg/L to 0.46875 mg/L were taken out of the fridge and before use, vortexed. Using a pipette, the bottom wells of the plate received 50 µL of sterile water, followed by the addition of BHI broth (150 or 200 µL, depending on the row and column) in the "controls" section. In the "bacteria" section of the plate, 150 or 200 µL of diluted bacterial inoculum (5 log CFU/mL) was subsequently introduced. Nanoparticles were handled with maximum carefulness and preciseness due to their potential instability under light and tendency to form sediment on the bottom of the tubes. Starting from the 1/32 dilution, 50 µL were used. Rows represent different concentrations of nanoparticle dilutions (ranging from 15, 7.5, 3.75, 1.875, 0.9375, and 0.46875 mg/L) along with 5 log CFU/mL of the bacteria inoculum. The plate was sealed with an adhesive plate seal.

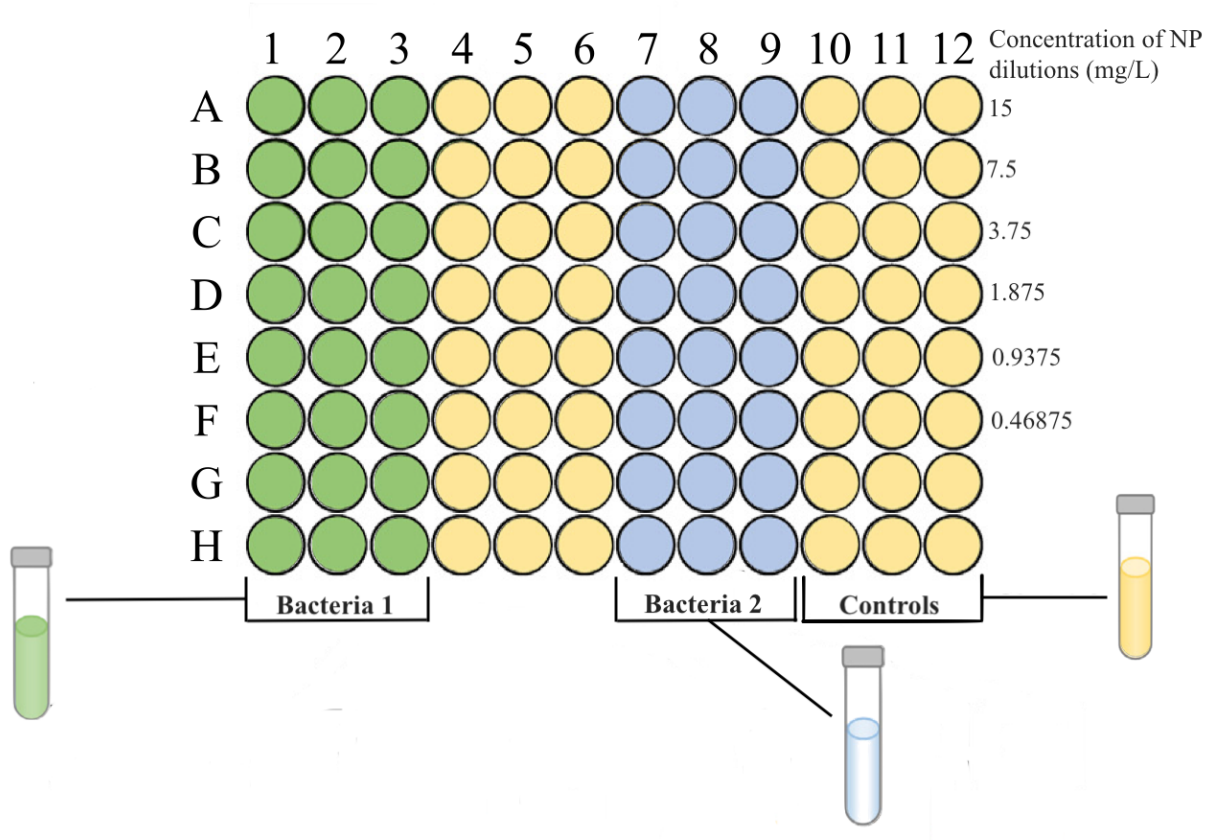

Figure 10: Microtiter plate (modified with Salam et al., 2023).

		1	2	3	4	5	6
50 ul of nanoparticle dilution + 150 ul bacteria or broth	A	15ml/l	15ml/l	15ml/l	15ml/l	15ml/l	15ml/l
50 ul of nanoparticle dilution + 150 ul bacteria or broth	B	1/2	1/2	1/2	1/2	1/2	1/2
50 ul of nanoparticle dilution + 150 ul bacteria or broth	C	1/4	1/4	1/4	1/4	1/4	1/4
50 ul of nanoparticle dilution + 150 ul bacteria or broth	D	1/8	1/8	1/8	1/8	1/8	1/8
50 ul of nanoparticle dilution + 150 ul bacteria or broth	E	1/16	1/16	1/16	1/16	1/16	1/16
50 ul of nanoparticle dilution + 150 ul bacteria or broth	F	1/32	1/32	1/32	1/32	1/32	1/32
200 ul bacteria	G	10^5 SA	10^5 SA	10^5 SA	BHI	BHI	BHI
50 ul water + 150 ul bacteria	H	Water+10^5SA	Water+10^5SA	Water+10^5SA	water+ BHI	water+ BHI	water+ BHI
		Bacteria			Controls		
		Nanoparticle					

Figure 11: MIC plate layout.

2.4.5 Spectrophotometer measurement

The optical density (OD_{600}) was measured by spectrophotometry using a Spectrophotometer Multiskan GO Microplate Reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The first reading was performed immediately after the plate preparation (T0), and the number of readings was set at 6 per 20 minutes. The microtiter plate was placed in the incubator at 37 °C for 24 hours on a shaker to allow bacterial growth and enable the evaluation of the NP's impact on inhibiting bacteria. Measurement of optical density (OD_{600}) was repeated (T24).

2.5 MIC determination and statistical analysis

We adopted a threshold of < 0.05 absorbance to determine the concentration that inhibited the increase of turbidity due to bacterial growth. This concentration was defined as the Minimum Inhibitory Concentration of the substance (Tsai et al., 2008). Negative control wells should not have any growth after incubation. If there is growth in the negative control part, the MIC values are not valid (Belanger & Hancock, 2021). MIC was determined by measuring the turbidity of the photometric device at 600 nm wavelengths.

Data were collected from spectrophotometer measurement and subjected to further analysis. The MIC was calculated by comparing the measurements at T0 and T24, using the aforementioned threshold (0.05). Mean, standard deviation, number of observations, and coefficient of variation (CV) were computed for each dilution/bacteria combination within each repetition. The mean indicated the average value, while the standard deviation showed the data variability. The number of observations represented the results' reliability. The CV measured the relative variability. A CV value ≤ 2.5 was considered acceptable. We assessed the intervention efficacy by testing the statistical significance of the differences in bacterial growth relative to the control groups.

3 Results

The outcomes of the antimicrobial activity tests of polyphenolic nanoparticles derived from pine are shown in the Table 2. Not applicable (NA) significates that the results did not align with the pre-set threshold of 0.05. It was found that the nanoparticle suspension tested exhibited various antibacterial actions against the selected pathogenic bacterial strains.

Table 2: Minimum inhibitory concentrations (MIC) of the polyphenolic nanoparticles extracted from pine against selected bacterial strains.

BACTERIA	log cfu/mL	MIC (mg/L)
E. coli	5	NA
M. haemolitica	5	3,75
P. multocida	5	15
S. aureus	5	NA
S. suis	5	NA
S. typhimurium	5	NA

NA = not applicable

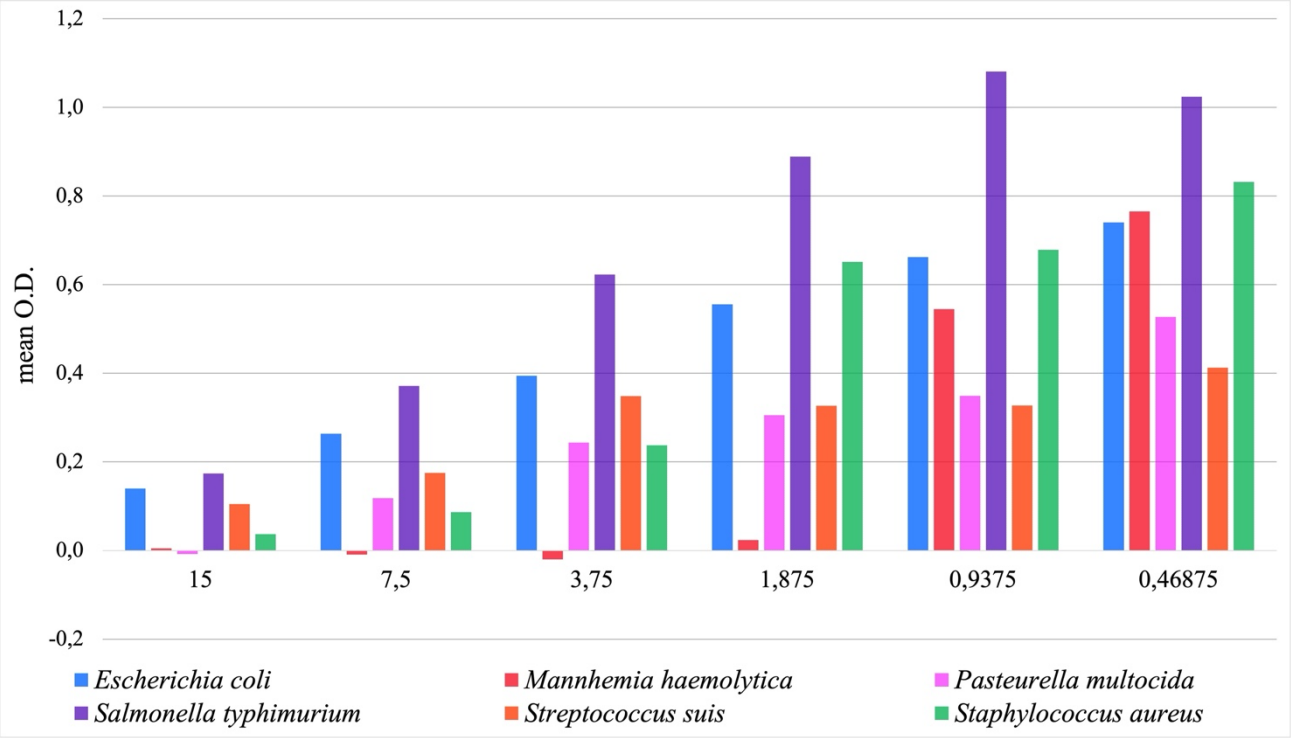

Figure 12: Different concentrations of NP's suspension versus bacteria tested (optical density) represented as a histogram.

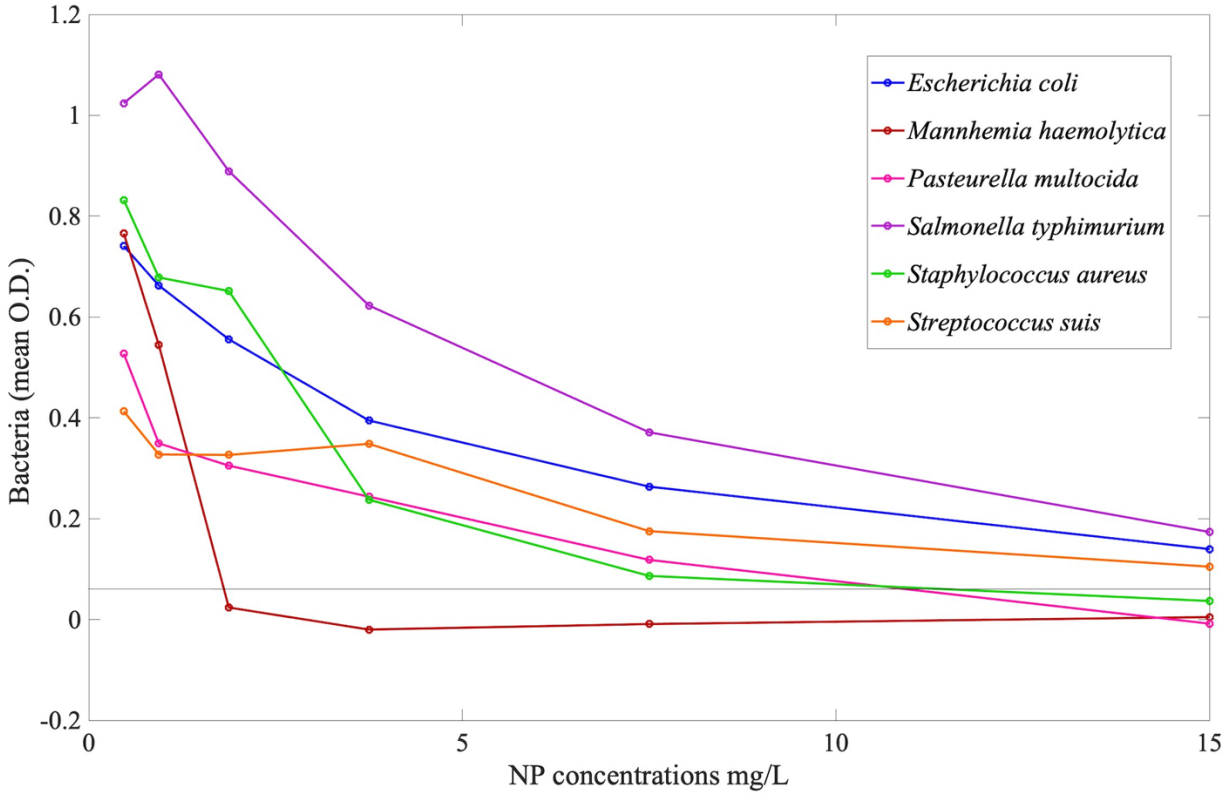

Figure 13: Different concentrations of NP's suspension versus bacteria tested (optical density) represented as a line plot.

Both graphs (Fig. 12 and Fig. 13) represent the inhibitory effect of polyphenolic nanoparticles derived from pine at different concentrations (mg/L) on the growth of selected bacterial strains. The optical density (O.D.) depends on the concentration of NP's suspension. The x-axis represents the NP concentrations (ranging from 0.46875 to 15 mg/L), while the y-axis represents the optical density of each bacterial strain. The values shown on the y-axis of the graph represent the mean of six independent replicates, calculated as the differences between the O.D. at T0 (immediately after preparation) and T24 (after incubation for 24 h at 37°).

From the graphs, it is evident, that the strongest inhibition occurred against *M. haemolytica* and *P. multocida*, followed by *S. aureus*, then *S. suis* and *E. coli*, while the lowest inhibition was observed against *S. typhimurium*. Considering the set threshold of 0.05, only *M. haemolytica* and *P. multocida* were successfully inhibited, using NP concentrations of 3.75 mg/L and 15 mg/L, respectively.

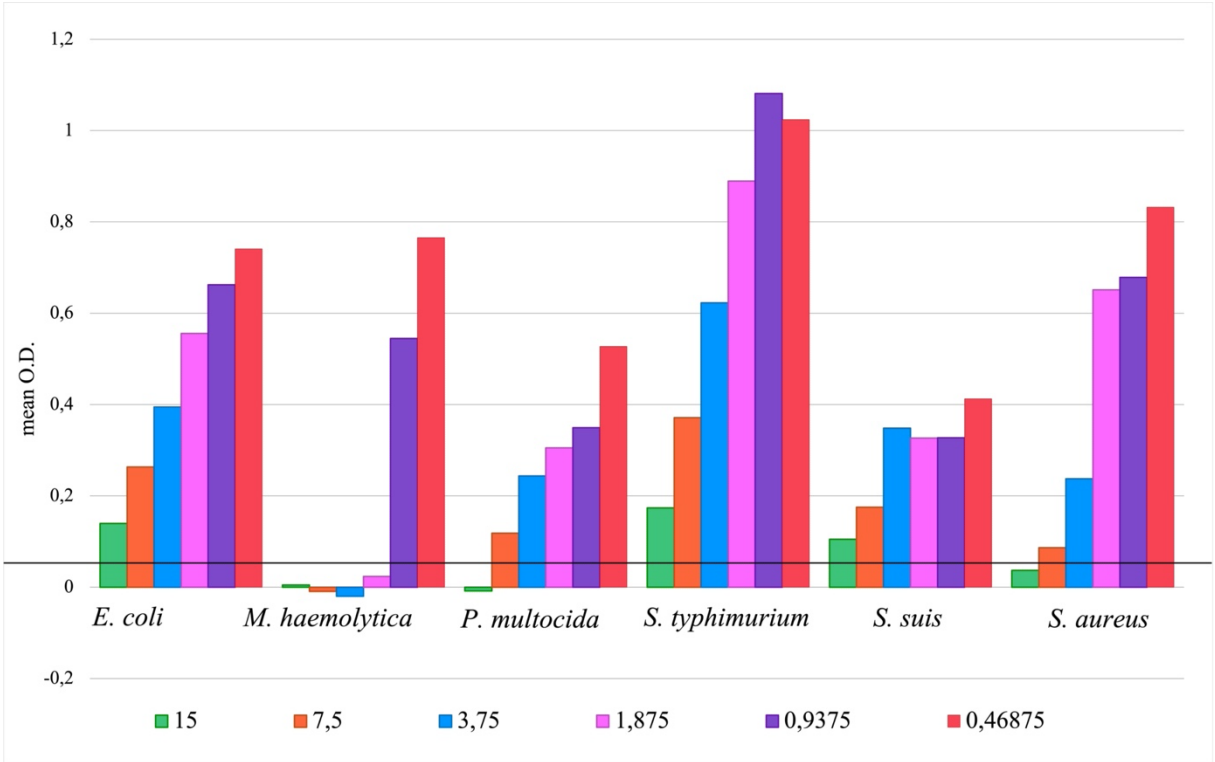

Figure 14: The inhibitory effect of polyphenolic NPs at different concentrations (mg/L) on the growth of various bacterial strains (O.D.)

When administering nanoparticle colloidal suspension of concentration 15 mg/L to selected bacteria, the final growth was overall the lowest, as expected (Figure 14). On the other hand, NP suspension of a concentration of 0.46875 mg/L was not notably efficient, in this case, *P. multocida* and *S. suis* showed the lowest counts. Even though the lowest concentration utilized (0.46875 mg/L) was not efficient in inhibiting any bacterial strain tested below the set threshold (0.05), the final values are still lower than those with no NPs administered. For example, the mean of final turbidity values of *E. coli* with the use of NP suspension of the lowest concentration was 0.7407. However, in the case of *E. coli* in broth (BHI) without NP addition, the final count was 1.1211.

4 Discussion

According to many literature sources (Dian et al., 2014; Huang et al., 2017; Liu et al., 2024; Shababdoust et al., 2020; Turuvekere Vittala Murthy et al., 2021) the use of polyphenolic compounds as feed additives brings certain disadvantages. Polyphenolic compounds tend to present low bioavailability, low water solubility, and overall low stability. Various types of nanoformulations for delivering polyphenols with antibacterial activity exist, which can also address the current limitations of polyphenols. As an example, it could be combated by using nanosized polymeric micelles (Dian et al., 2014), polyphenols-loaded nanofibers (Shababdoust et al., 2020), or nanocarriers (de Almeida Roger et al., 2018; Huang et al., 2017). Implementation of nanosized particles offers many advantages, like good polyphenols' stability, their protection against oxidation, and the charge on the surface which creates stable colloidal suspension. Their small size, high surface area, and improved permeability are important characteristics in their use as antimicrobial agents (Fatima et al., 2021). This high surface-to-volume ratio and specific surface area enable nanoparticles (NPs) to have an exceptionally high contact area compared to molecular materials of the same mass. Molecules such as antibiotics interfere with specific bacterial processes having specific targets, whereas NPs act through more mechanisms simultaneously and they affect multiple cellular components at once (Mamun et al., 2021).

All the above-mentioned nanoformulations (polymeric micelles, nanofibers, nanocarriers) share one downside, the manufacturing of such compounds can be costly. In our study, a waste product from the wood industry displaying nanomaterial features such as size and colloidal stability was considered for its abundance and availability. Indeed, the polyphenolic NPs in this study were unintentionally created for other purposes, from an industrial process that can be meant as a top-down nanoparticle production. Six strains of pathogenic bacteria from various families were investigated for their susceptibility to these polyphenolic NPs. These included *Pasteurellaceae* (*Pasteurella multocida* and *Mannheimia haemolytica*), *Enterobacteriaceae* (*Escherichia coli* and *Salmonella typhimurium*), followed by *Staphylococcaceae* (*Staphylococcus aureus*) and *Streptococcaceae* (*Streptococcus suis*). The bacteria tested are known to use various AMR (antimicrobial resistance) mechanisms. *E. coli* can produce toxins known as extended-spectrum-beta-lactamases (ESBL) (Laconi et al., 2023) which inactivate critically important antibiotics. *E. coli, S. aureus*, *S. typhimurium*,

and many other important zoonotic pathogens form biofilms (Aleshukina et al., 2020) which are considered to be highly resistant to antimicrobial agents (Ito et al., 2009), and their formation is inevitably related to the transfer of antimicrobial resistance genes (Laconi et al., 2023). *S. aureus, E. coli* (Blair et al., 2015; Kapoor et al., 2017) and *P. multocida* (Lin et al., 2022) have the ability to alter the drug targets which prevents them from functioning normally, consequently antibiotics cannot bind to them. Efflux pumps are the primary mechanism during the initial stages of an antimicrobial compound's resistance development (Costa et al., 2013). *E. coli* (Soto, 2013), *P. multocida* (Lin et al., 2022), and *S. typhimurium* (Peng et al., 2018) are known to present efflux pump resistance mechanism, as well as *S. aureus* in which efflux pumps represent an important contributor to fluoroquinolone resistance (Costa et al., 2013).

The polyphenolic nanoparticles (NPs) tested in this study exhibited potent and selective antibacterial activity against the family *Pasteurellaceae* (*P. multocida* and *M. haemolytica*). This finding is significant, given the high relevance of these bacteria in the context of antimicrobial resistance (AMR). According to Ziagham et al. (2024), *P. multocida* proved to be resistant to several antibiotics (ampicillin, clindamycin, lincomycin, streptomycin, penicillin) making this bacterium candidate of multi-drug resistant (MDR) bacteria. Diverse virulence factors have been connected with *Pasteurella*'s pathogenicity, like capsules, adhesins, toxins, or siderophores (Ziagham et al., 2024). Both *P. multocida* and *M. haemolytica* cause respiratory disease in livestock animals and *P. multocida* can be transmitted to humans as well (Dabo et al., 2008; Punpanich & Srijuntongsiri, 2012). Melchner et al. (2021) concluded that multi-drug resistance is more prevalent in *P. multocida* than in *M. haemolytica*, and the highest resistance was present to antibiotics such as aminocyclitol and tetracycline. Strains belonging to the *Pasteurellaceae* family are known to develop or acquire resistance genes or resistance-mediated mutations using plasmids, transposons, or integrative and conjugative elements that carry resistance genes. Since it involves horizontal gene transfer, it can occur not only between bacteria of the *Pasteurellaceae* family but also with other Gram-negative bacteria (Michael et al., 2018). In addition to that, it is relevant to note that horizontal gene transfer is the main mechanism for the spread of antibiotic resistance (Džidić et al., 2008)

Some of the mechanisms of antibacterial activity mediated by polyphenols are the following: inhibition of virulence factors, direct action on a microbial cell wall, inhibition of bacterial enzymes, oxidative stress, inhibition of bacterial gene expression, and anti-biofilm activity.

Some of these are non-specific (oxidative stress), while other mechanisms only act in certain bacteria (toxin inhibition) (Renzetti et al., 2020; Turuvekere Vittala Murthy et al., 2021).

Specifically, Villanueva et al. (2023) demonstrated an anti-biofilm activity of selected tannins in *S. typhimurium*, *Pseudomonas aeruginosa*, *E. coli*, and *S. aureus*. The polyphenolic NPs tested exhibited not only bacteria-specific activity but also inhibitory action against different bacterial species. Because biofilm creation is a common behavior of many bacteria from different families, polyphenolic NPs could be explored for their possible use as a biofilm disruptor.

Several research groups (Chapa González et al., 2023; Sathiyaraj et al., 2021) explored different types of nanoparticles for microbial inhibition with outcomes suggesting overall increased efficacy to Gram-negative bacteria. The responsible factor is thought to be the difference between the membrane composition of these two groups, Gram-positive bacteria have a thick and exposed peptidoglycan layer. In the case of this study, the highest efficacy was against Gram-negative bacteria (Pasteurellaceae). However, a reduced antibacterial effect was also noted against Gram-positive bacteria (*S. aureus* and *S. suis*).

Combining nanoparticles with conventional antibiotics has the potential to both mitigate the spread of microbial resistance and enhance the effectiveness of previously ineffective antibiotics. NPs can deliver antibiotics and help them penetrate bacterial cell walls. The antibiotics then damage the cell walls, making it easier for the NPs to enter. Nanoparticle-antibiotic complex created brings strong synergism against pathogenic bacteria (Mba & Nweze, 2021).

Agro-industrial wastes occurring are often rich sources of bioactive compounds (phytochemicals) (Fierascu et al., 2019). Re-utilizing the wood waste object of this research is a sustainable and cost-effective method for producing novel feed additives for livestock production. By converting industry by-products into valuable resources, we can enhance livestock health and growth while promoting environmental sustainability and reducing production costs.

5 Conclusion

The results provided insights into the efficacy of polyphenolic nanoparticles and their potential use as feed additives in livestock production. MIC assessment revealed that polyphenolic nanoparticles exhibit significant inhibitory effects against some of the selected bacterial strains, including those frequently resistant to antibiotics. *M. haemolytica* and *P. multocida* showed susceptibility to the tested nanoparticle, while *E. coli*, *S. suis S. aureus*, and *S. typhimurium* growths were unaffected by the compounds. Our findings could help to mitigate antimicrobial resistance in farm animals. Embracing the One Health approach is essential, as it not only addresses animal health but also contributes to improving antimicrobial resistance management across human populations and the environment.

Further research is needed to perform *in vivo* experiments and field applications. The nanoparticle's safety profile should be assessed, as well as understanding the correlation between chemical-physical properties and antimicrobial activity is of primary importance. Their combined effect with other polyphenolic nanoparticles coming from a different source should be explored, as well as with conventional antibiotics as it was suggested by many research groups mentioned in my project. This combined therapy could represent an alternative to antibiotics or help restore the antibiotic's activity despite pre-existing antimicrobial resistance.

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