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Review Article

A Systematic Review of Essential Oils' Antimicrobial and Antibiofilm Activity against *Klebsiella pneumoniae*

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Abstract

Biofilms are sessile microbial life forms recognized for their surface adherence and the formation of extracellular polymeric substances consisting of a gluey network that surrounds the cells. Biofilms can be produced by bacteria such as *Klebsiella pneumonia*. Several studies investigate strategies, including the use of essential oils to detain, reduce or even eliminate their formation, especially in hospital medical supplies. The present study performed a systematic review among different databases about the use of essential oils in the control of *K. pneumoniae* biofilm formation. Because of this bacteria's proclivity for acquiring resistance, the selected research shows that this is a good model. The search for alternative techniques to manage these diseases is extremely important because a large proportion of bacteria do not react to traditional therapies, making control impossible. The current study focuses on high-impact published studies and show that essential oils are efficient against *K. pneumoniae*, mainly because of their resistant nature. Because certain bacterial strains do not respond to standard therapies, the hunt for new approaches to control this infection is critical. After an extensive search in the literature, a high number of studies related to this topic were found, from which we selected 120 among all.

Keywords: Biofilms; Essential oils; Hospital medical supplies; *Klebsiella pneumoniae*

Introduction

Klebsiella pneumoniae is a gram-negative bacteria, considered an opportunistic pathogen because it can grow in a wide range of hosts and environments. Over in the past years, there has been an increase in infections caused by resistant *K. pneumoniae* strains, leading to serious consequences, especially in hospital areas, such as longer hospitalization time, the absence of therapeutic options, and also an increase in morbidity and mortality numbers [1]. Normally, the infections caused by *K. pneumoniae* are asymptomatic and can reach the gastrointestinal tract, the skin, the nose, and the throat of healthy individuals [2]. The transmission occurs mainly through hand contact. Nevertheless, when there is a homeostatic imbalance, this microorganism can cause a variety of infections depending on the site, generating urinary and respiratory infections but also more severe complications in the blood as well as hepatic abscess, meningitis, and sepsis [3]. The infection can be transmitted mostly through direct or indirect contact between patients, health professionals, contaminated objects, visitors, or travelers [4]. In hospitals, for example, the high number of people circulating in an enclosed area leads to possible bacterial resistance. In addition, the absence of hand sanitization from professionals and visitors contributes to the dissemination of these resistant strains in different places, such as public transport, supermarkets, beaches, and parks [5]. In this sense, there are several factors involved in the dissemination of *K. pneumoniae* in a hospital environment. Considering that this bacteria is an opportunistic microorganism, and it possesses a great ability to produce biofilms, and it has been the subject of extensive studies in the development of nosocomial infections [6]. Among these, catheters use, time of stay in these environments, long hospitalization time in the Intensive Care Unit (ICU), prolonged antimicrobial therapy, reutilized instruments in surgeries and implants, and/or devices can all provide open access to *K. pneumoniae* infections [7,8]. Susceptible individuals such as diabetics, transplanted patients, and people with compromised hepatic function and dialysis patients are among the group at risk considering their immunodeficiency, which results in a defect in the immune system that can no longer work properly against the infection. This immunodeficiency is a side effect of the therapies as a consequence of their treatment, considering that in these conditions, the immune system does not work properly [4,9]. Biofilms are classified as a microbial community that sits in a thin layer of either biotic or abiotic surfaces, established through the extracellular polymeric substances that originate from the biofilm itself. *K. pneumoniae* biofilm formation has been linked to an important pathogenic feature of this bacteria, particularly in the use of medical catheters. Biofilm matrix provides the necessary conditions to mitigate drug delivery regarding its dense polysaccharide and protein layer in which the antibiotic can't diffuse properly, resulting in significant exposure to bacteria and, therefore, the establishment of chronic infections in the urinary tract [4, 10, 11]. Alternative methods are being studied to minimize the consequences caused by biofilm formation. Among these methods is the research on the activity of antibiofilm formation of essential oils (EOs) derived from plants. EOs are a viable therapy option for contaminated patients who have been infected with resistant strains. There has been a tremendous surge in research on aromatic and medicinal plants in recente years [12-22]. However, to obtain an adequate and commercial formula, there are a few factors to consider, such as geographical morphology, climate, and cultural conditions [13]. The primary purpose of this study is to conduct a systematic review of the usage of essential oils in the administration of *K. pneumoniae* biofilms using scientific databases.

Materials and Methods

The current study was undertaken using a bibliographic search of PUBMED, MEDLINE, Scientific Electronic Library Online (SCIELO), Academic Google, and other relevant websites, from 2012 to 2022. "Biofilm," "*K. pneumoniae* and biofilm," "biofilm and medical catheters," "biofilm and essential oils," and "*K. pneumoniae* and essential oils" were the top search terms because they addressed the study's major goal. The initial search was undertaken by reading the titles and abstracts and only full and published papers were chosen to participate in this review follows the careful reading.

Results and Discussion

The keyword "biofilm" was the most comprehensive among all others regarding the present study in our search. From the period selected, there were 100,951 published papers, whilst for the term "*Klebsiella pneumoniae* and biofilm," 10,979 studies were found. 7.387 publications were found for the term biofilm and medical catheters, and for the term "biofilm and essential oils," there were 42 studies. As for the term "*Klebsiella pneumoniae* and essential oils," only 20 publications were found. These results are presented in Table 1 and suggest that the majority of the studies are not focusing on the main goal of this review.

Search term	Number of published papers between 2012 and 2022
Biofilm	100,951
K. pneumoniae and biofilm	10,979
biofilm and medical catheters	7,387
biofilm and essential oils	42
K. pneumoniae and essential oils	20

Table 1: Number of published papers in the last 10 years according to the search term

This review included the main studies discovered through the selected databases. The relevance of biofilm formation was stated in the work of Bergamo G, et al., in which the author observed a significant increase in the number of publications since 1983 [23]. These data showed that biofilms had been a relevant subject over the years for the scientific community and the need for further studies to identify the causes and possible solutions for this matter.

Use of essential oils in *K. pneumoniae* strains

Total number of studies found correlating the use of EOs in *K. pneumoniae* strains was 20. As of today, *K. pneumoniae* is the most nosocomial pathogen that carries the carbapenemase gene (KPC) that gives resistance to carbapenem, a classic beta-lactam antibiotic that is used for the treatment of bacterial infections. As a result, the search for novel antimicrobials is necessary. Natural products, such as EOs, are a promising source due to their complex composition. These products are demonstrated to be effective against resistant strains, as shown in Figure 1, but the mechanisms underlying their actions are not fully understood.

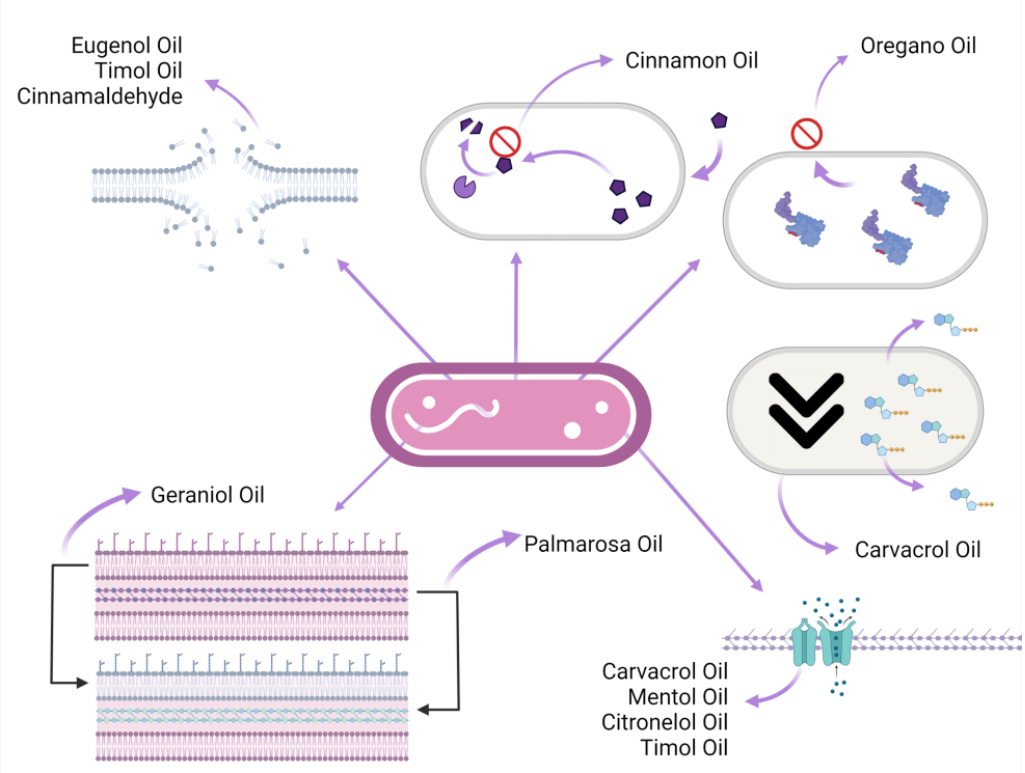

Figure 1: EO action sites and mechanisms in a bacterial cell

Eugenol promotes disruption of the cytoplasmic membrane, increasing its nonspecific permeability, causing ion leakage and excessive loss of other cellular components, including intracellular proteins, leading to cell death. Geraniol, linalool act on the wall, changing permeability and modifying membrane proteins and periplasmic enzymes, as well as altering the membrane, ion transport, and energy production. Palmarosa oil penetrates the lipophilic part of the cell membrane generating na imbalance in the membrane potential and losing its selective permeability. The compounds methyl carvacrol, menthol, citronellol, and thymol also cause a widening of the cell membrane that leads to the passive diffusion of ions between the phospholipids. They also act on the cell wall of bacterial cells, causing rupture and extravasation of the extracelular contents. Carvacrol induces leakage and loss of ATP from bacterial cells. Cinnamaldehyde inhibits ATPase enzymes and disrupts the outer cell membrane.

A better understanding of the molecular mechanisms of EOs when used as an antibacterial agent against KPC-*K. pneumoniae* could lead to improved clinical efficacy. According to Yang SK, et al., the proteomic perspective was investigated toward KPC-*K. pneumoniae* cells when comparing the untreated cells with cells treated with cinnamon essential oil (*Cinnamomum verum* J. Presl) (CBO) [16]. The authors reported that cells exposed to CBO express a higher level of oxidative stress tht eventually tears the bacterial membrane, possibly by interacting with the lipid bilayer.

Interestingly, different repair membrane pathways were affected by oxidative stress and hence contributed to cell viability loss. Iseppi R, et al. investigated the antibacterial activity of *Cinnamomum cassia L* [19]. (CCeo) isolated and in combination with polymyxin B (a comercial antibiotic) against carbapenemase-producing *Klebsiella pneumoniae* and *Serratia marcenscens.* The association between CCeo and polymyxin B showed a reduced number of viable cells in a 4-hour treatment. When associated with polymyxin B, a synergistic effect was displayed, and the bacterial growth was rapidly inhibited, demonstrating that the CCeo is a promising candidate in the development of an alternative method for carbapenem-producing strains. *Cinnamomum verum* essential oil has been used as an alternative treatment for different diseases. Wijesinghe GK et al. showed the antibacterial and antibiofilm properties of essential oil leaves from *Cinnamomum verum* against, *K. pneumoniae, P. aeruginosa,* and *S. aureus* [24]. All tested strains displayed sensitivity towards cinnamon oil steamer. Cinnamaldehyde are one of the major compounds in cinnamon EO, and its concentration ranges between 60 and 80% when obtained from the bark of the *Cinnamomum* genus [25]. Different studies demonstrate the antimicrobial activity of cinnamaldehyde against different bacteria, including *K. pneumoniae, L. monocytogenes, P. aeruginosa, E. coli, E. faecalis, S. aureus, S. epidermis, V. parahemolyticus and Salmonella* spp. [25].

Dhara L, et al. tested the cinnamaldehyde and eugenol antibacterial activity against Enterobacteriaceae ESBL quinolone-resistant (QR) strains along with their *in vivo* toxicity in a murine pharmacological model [26]. In this study, the authors observed the high efficiency of cinnamaldehyde and eugenol as promising antibacterials with no considerable toxicological and behavioral effects. Anandhi P, et al. add that wound infection is a great site for pathogenic microorganisms [27]. Cinnamon and clove oil are effective antibacterial agents due to their ability to reduce virulence and pathogenicity in a wound healing *in vivo* model against *K. pneumoniae, P. aeruginosa,* and *E. coli.*

The ethanolic extract (CEE, 0,01-1 mg/plate), essential oil (CNO, 0.125-1 mg/plate) from cinnamon bark, and cinnamaldehyde (CLD, 0,125-1 mg/plate) were eficiente against different pathogens, such as *K. pneumoniae, E. coli*, *S. aureus, Proteus vulgaris,* and *S. typhimurium (S. typhimurium)* in an agar diffusion protocol. CEE and CLD showed promising antimutagenic and antimicrobial properties, respectively. Therefore, their properties should be explored in depth in an *in vivo* condition.

Aromatic herbs such as oregano (*Origanum vulgare*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*) is used for ornamental, culinary, and phytotherapy purposes all over the world. The antibacterial activity of these plants has attracted the scientific community's interest as a potential alternative to conventional antibiotics in addressing microorganism resistance. There has been a tremendous increase in the growth of several aromatic and therapeutic plants in recent years. Fournomiti M, et al. showed that *K. pneumoniae* displayed a higher sensitivity towards thyme oil, followed by oregano, whilst sage oil did not present any antibacterial activity [13].

Souza ERL, et al. tested the hybrid *Lavandula* essential oil against *K. pneumoniae* strains, in which the authors observed a moderate antibacterial activity along with a bacteriostatic property [28]. These results were in agreement with Kozics K, et al., which confirmed the strong antibacterial activity of oregano (*Origanum vulgare*), sage (*Salvia officinalis)*, thyme (*Thymus vulgaris*), arbovitae (*Thuja plicata*), Cassia (*Cinnamomum cassia*), lemongrass (*Cymbopogon flexuosus*) against *P. aeruginosa, P. vulgaris, C. koseri* and *K. pneumonia* [15]. The enzyme extended-spectrum beta-lactamase (ESBL) is synthesized by the Enterobacteriaceae family, which includes *K. pneumoniae* and *E. coli*. Penicillin, cephalosporin, cephamycin, and carbapenem are among the beta-lactam antibiotics that the ESBL can hydrolyze. The bacteria that produce ESBLs spread predominantly from ranches to slaughterhouses and human meals derived from animals.

Ginting EV, et al. discovered that *Syzygium aromaticum* (clove) and *Cinnamomum verum* (cinnamon) have effective antibacterial action against resistant *K. pneumoniae* and *E. coli* ESBL-strains

[21]. Iseppi R, et al. analyzed the antibacterial effects of four different EOs, *Melaleuca alternifolia, Eucalyptus globulus, Mentha piperita,* and *Thymus vulgari* as an alternate method to prevent the progress and dissemination of *K. pneumoniae* and *E. coli* ESBL-strains, Metallo beta-lactamase (MBL)-producing *P. aeruginosa* and KPC-producing *K. pneumoniae. M. alternifolia* and *T. vulgaris* EOs presented the most effective antibacterial activity in all tested strains, with *M. alternifolia* as the best candidate even at low concentrations [18].

The antibacterial activity of oregano (*Origanum syriacum* L.), thyme (*Thymus syriacus* Boiss.), cinnamon (*Cinnamomum zeylanicum* L), and clove (*Syzygium aromaticum*) EOs were the most effective among 28 different EOs in a study performed by Al-Mariri A, et al. against four different Gram negative strains, *K. pneumoniae*, *E. coli* O157:H7, *Yersinia enterocolitic* O9 (*Y. enterocolitica* O9) and *Proteus* spp [29].

The authors compared their efficiency with that of cephalosporin and ciprofloxacin, two well-established antibiotics that were effective. The discussed studies demonstrate promising antibacterial activity against *K. pneumoniae*, especially for its resistance to conventional treatments. As a result, it is necessary to discover new alternative methods to control the spread of antimicrobial-resistant strains.

Elements of biofilm formation in a hospital environment

One of the causes for researching bacterial biofilm formation in medical devices, as evidenced by the papers chosen for this study, is that they are responsible for a high infection rate, accounting for roughly 80% of human illnesses.

Bacterial resistance has been a major factor in the high rate of hospital infections worldwide [30, 31]. There has been an increase in the mortality rate, demonstrating the seriousness of biofilm formation and the need for a clean hospital environment, contributing to the reduction of health costs and also patient quality of life.

This the investigation is essential considering that the microorganism's ability to develop a biofilm is directly proportional to its aptitude to cause na infection. Research conducted by Ferreira ACB, et al. showed the risk of infection in catheters in hemodialysis patients in Brazil [30]. Several other studies also demonstrated a high number of infections in patients with catheters and prosthetic devices [31-37].

Biofilm formation is a prokaryotic adaptation used by several microorganisms, called resistant microorganisms, that have developed a growth strategy to overcome antibiotics and vaccines. These evolutionary steps allow the survival of organisms in hostile environments and the colonization of new sites through dispersion mechanisms [23]. As a result, these microorganisms acquire strong intercellular communication and can rapidly respond to subtle changes in their surroundings through a signaling mechanism called *quórum sensing* [38].

Among the several microorganisms that can produce biofilm are *K. pneumoniae, P. aeruginosa*, *E. coli, S. aureus*, *S. epidermidis,* and *C. albicans. K. pneumonia* is a gram-negative bacteria and an important opportunistic pathogen that can cause several health problems, including tract infections, bacteremia, pneumonia, and hepatic abscesses. There is an alarming situation regarding multi-resistant (MDR) and hypervirulent (hvKP) *K. pneumoniae* strains, whose mechanisms are not yet fully understood. Therefore, it is necessary to elucidate the pathogenic and resistance pathways of this microorganism [39].

Bergamo G, et al. discussed that microorganism resistance is related to poor penetration from antimicrobial agents to the polysaccharide membrane in which the bacteria develop the biofilm [23]. Assays that can evaluate both the action and properties of antimicrobials against biofilms are of great value the minimizing infection rates. Several other factors can influence biofilm formation, such as altered bacterial growth and gene expression [41-43].

The well-ordained tridimensional biofilm structure is a result of different molecular associations compared to Other non-sessile microorganisms. Among the molecules expressed on the biofilm surface that allow substrate adhesion and, consequently, an elaborated structure are fibrinogen-binding proteins, fibronectin, capsular polysaccharide-adhesion factors, autolysin, and teichoic acid. This explains the antibiotic selectivity towards bacterial strains and the lack of capacity to eliminate biofilm formation from resistant microorganisms. In addition, virulence factors and nutrient optimization contribute to higher antibiotic resistance [41].

During biofilm formation, the microorganisms can be found in a progressive accumulation phase of virulence factors, increasing the risk of infection in patients. As a result, research on biofilm formation in a medical environment must be kept up to date for academic and scientific communities [38, 40].

Biofilm formation in hospital catheters

Biofilm formation in hospital catheters is a serious worldwide health problem because it is responsible for triggering several infectious diseases [44]. Figure 2 shows different catheters and probes used in hospitals daily.

Figure 2: Catheters and probes that are used daily in hospitals

Contamination of urinary catheters, for example, can result in urinary infections [44]. Likewise, a central venous catheter can also present an infection. This demonstrates the subject's relevance to ensuring a high-quality service from hospitals and providing a better quality of life for patients [44,45,46].

Viega PIM highlights that catheter-associated urinary infections are common in patients with long urinary catheterization periods [47]. The factors related to biofilm formation are crucial for choosing the best intervention strategy to minimize the risk of infection. Furthermore, contamination of a central venous cateter is very concerning because microorganisms can circulate throughout the entire organism, exacerbating an already severe infection [44-46].

Central venous catheters are widely used in hospitals because they provide conveniente access to a patient's vascular system. As a result, it allows for the administration of drugs and other vital components, particularly in hospital circumstances [48].

On the contrary, urinary infections are responsible for high morbidity and mortality rates [40]. One of the main causes of infections in hemodialysis patients is material manipulation by health professionals [30,49]. The use of urinary catheters is essential in patients where access to the arteriovenous fistulae is not possible and, or access to the blood needs to be fast [49].

The use of urinary catheters is associated with a high infection rate as a result of biofilm formation **(**Figure 3**)**, restricting the benefits that they could provide for patients [49,50]. On catheter surfaces, the most reported microorganisms are *S. aureus*, *S. epidermidis*, *P. aeruginosa, K. pneumoniae,* and *C. albicans* [49,51].

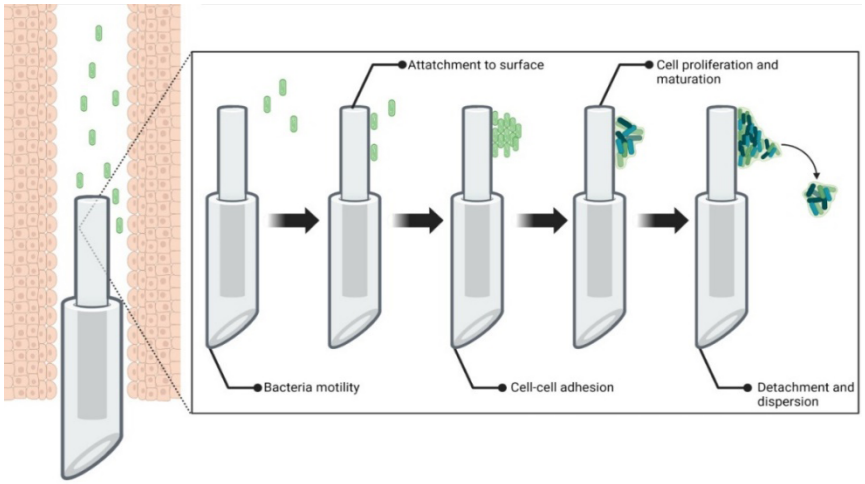

Figure 3: Biofilm formation in hospital medical equipment

Use of essential oils in biofilms

Bacterial biofilms are associated with high resistance against several antimicrobial agents, contributing to persistent microbial infection and, consequently, therapy failure. Bilcu M, et al. tested the efficiency of vanilla (*Vanilla planifolia*), patchouli (*Pogostemon cablin* syn. *P. patchouli, P. heineanus*), and ylang-ylang (*Cananga odorata* subsp. *Genuine)* EOs stabilized by Iron Oxide-C14 nanostructures against *K. pneumoniae* and *S. aureus* biofilms [12].

The vanilla nanoparticles created a thin coating layer on the catheter's surface that strongly inhibited both the initial 24h-adherence from *S. aureus* and the 48h-development. Patchouli and ylang-ylang EOs nanoparticles primarily inhibited the initial adhesion. All nanostructures showed similar efficiency toward *K. pneumoniae* biofilm, presenting a new antibiofilm and anti-adherent coating-surface design.

Mohsenipour Z, et al. studied the effects of *Thymus vulgaris* against the planktonic form of six clinical isolates of pathogenic bacteria and their biofilm structures, *S. aureus*, *B. cereus,, S. pneumoniae, P. aeruginosa, E. coli,* and *K. pneumonia* [14]. According to MIC and MBC concentration results, *Thymus vulgaris* extracts were efficient at inhibiting bactéria in their planktonic form. In addition, antibiofilm formation behavior displayed a concentration-dependent behavior.

Thymol and piperine are two bioactive compounds with several pharmacological activities, such as Ndezo B, et al., evaluated their antibiofilm capacity, isolated or in combination with three aminoglycoside antibiotics (streptomycin, amikacin, and kanamycin) against *K. pneumoniae* biofilm [22]. The results showed that thymol, in combination with antibiotics had a synergistic effect in both biofilm formation and biofilm destruction, implying a possible alternative therapy for *K. pneumoniae* biofilm-related infections.

Recently, Dudek-Wicher R, et al. performed a comparative study between the antimicrobial activity of liquid and volatile fractions from five EOs, *Syzygium aromaticum* L., *Boswelia serrata* Roxb., *Juniperus virginiana* L., *Pelargonium graveolens* L., and *Melaleuca alternifolia* Cheel., against six planktonic and biofilms from *S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa, E. coli*, and *C. albicans* [20]. The results contribute to EOs benefits, demonstrating the ability to fight biofilm-forming pathogens.

A preliminary study demonstrated eugenol's antibacterial activity against *K. pneumoniae*. However, its mechanism of action remains unexplored, Qian W, et al. investigated the antimicrobial activity of eugenol against carbapenem-resistant *K. pneumoniae* strains [52]. Eugenol was able to inhibit biofilm formation and inactivate the carbapenem-resistant *K. pneumoniae* (CRKP) cells. Moreover, eugenol displayed strong inhibitory effects on biofilm formation and gene expression associated with it. These findings suggest that eugenol possesses a strong antimicrobial effect and can potentially be used to control CRPK-related infections.

Cinnamomum verum EO has been used as a therapeutic alternative for several diseases, Bassetti M, et al. studied the antibacterial and antibiofilm properties of this EO against *K. pneumoniae, P. aeruginosa, and S. aureus* [53]. The results were promising, considering the *Cinnamomum verum* EO did not display cytotoxicity in HaCat cells for any tested concentrations. Furthermore, according to phytochemical analysis, eugenol was the most abundant component in the EO.

Kwiatkowski P, et al. performed a study to evaluate the antibacterial and antibiofilm activities of selected constituents from EOs against NDM-1-producing uropathogenic *K. pneumoniae* strains [54]. The results provide a more detailed analysis of the phytochemicals in their application against *K. pneumoniae*.

Pimpinella anisum L., Cinnamomum zeylanicum, Syzygium aromaticum, and *Cuminum cyminum L.* EOs have antibacterial action against planktonic and biofilms of *K. pneumoniae*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, *A. hydrophila*, *P. mirabilis*, and *C. albicans*. Cinnamon and clove displayed the best results among all tested EOs. When analyzing biofilms, *Cinnamomum zeylanicum* appears to be the best EO to inflict a disturbance on gram-negative bacteria biofilms [55].

These studies demonstrate the efficacy of EOs toward microbial biofilms and resistant bacteria. Besides, it is important to mention that all selected publications showed promising results and can be important tools as alternative methods to control these microorganisms.

***K. pneumoniae* gene influence in biofilm formation**

Jacobsen AL, et al. demonstrates that bacterial fixation in catheters is initiated by adhesins, such as fimbriae, presented on the bacterial surface [56]. *K. pneumoniae* produces mainly two types of fimbriae: type 1 and type 3 [57].

These two fimbriae are mechanically different because of their functions [58]. These fimbriae are made up mostly of protein primary structure monomers, with an average molecular weight of 20 kDa and adhesin subunits.

The main monomeric structures in type 1 and type 3 fimbriae are FimA and mrkA, respectively. The main adhesin subunits, fimH or mrkD, can be coded by chromosomic and plasmodial genes [58,59]. Type 1 fimbriae are mannose-specific and are expressed in several enterobacteria, whereas type 3 fimbriae are specific for

collagen IV and V [6,60].

Type 1 fimbriae promotes adhesion and bacterial invasion along with biofilm formation, which is characteristic of bacterial virulence [61]. As seen in Figure 4, both type 1 and type 3 fimbriae are expressed during biofilm formation. However, according to some authors, type 3 fimbriae contribute to biofilm formation [62], but type 1 doesn't [63].

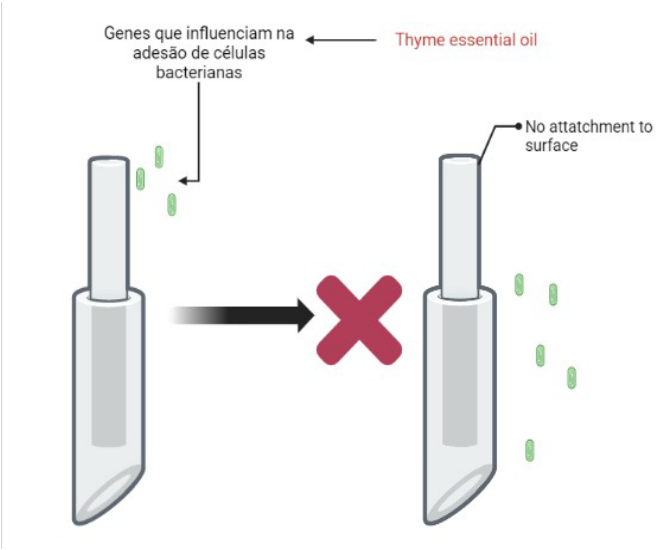

Figure 4: Structural representation and possible genes involved in biofilm formation and adhesion of *K. pneumoniae.*

The fimbriae relation *to K. pneumoniae* biofilm formation has already been confirmed, but more studies are necessary because it has not been clarified which genes are involved in this process. There are a few examples regarding other microorganisms that can help to understand, including *P. aeruginosa, C. albicans, E. coli,* and *S. aureus*.

The initial phase (0–11h) of *C. albicans* biofilm formation occurs initially with yeast adherence to the substrate, followed by cell coaggregation and colonization (0-11 h) [42,65]. The presence of yeast is critical for surface anchoring. Then, growth and proliferation occur, with the development of the basal layer, cell anchoring, and production of the extracellular matrix material, as the intermediate phase (12-30 h). The maturation phase occurs between 31 and 72 h, in which the pseudohyphae grow and mature, followed by hyphae extension from germinative tubes and dissemination of cells [42,65,66].

Molecular mechanisms of biofilm regulation have already been described by a few authors [42,67,68]. The first step is adherence, which occurs through the *ALS* gene family, expressed by yeasts and hyphae, and responsible for adherence and aggregation to other microorganisms. The HWP1 gene encodes a hyphae-adhesin only, whereas the EAP1 gene grants adherence to polystyrene and the CSH1 gene confers superficial hydrophobicity adherence, along with other lesser-known adhesins [42,69] Figure 5.

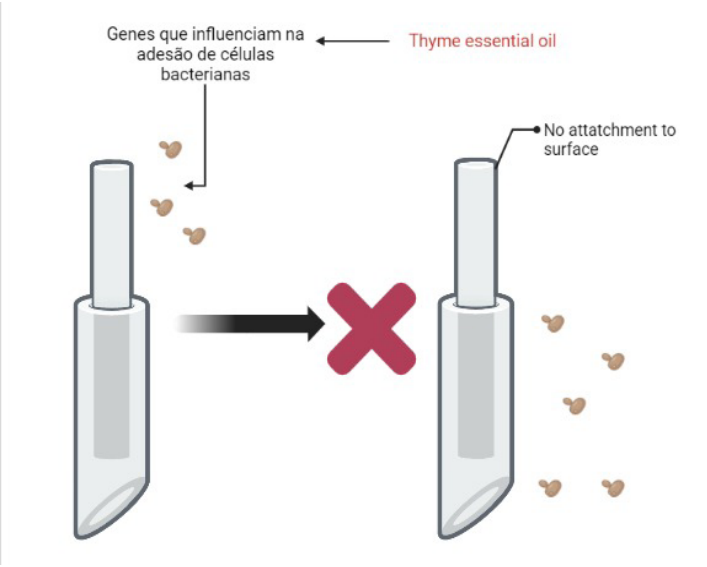

Figure 5: Structural representation and possible genes involved in biofilm formation and adhesion of *C. albicans.*

Adherence is followed by maturation, with hyphae formation, and the EFG1 gene is involved in morphological transition regulation and can produce an adherent structure to polystyrene, polyurethane, and glass. Other genes are also required for morphological differentiation, such as CPH1, TEC1, SUV3, NUP85, UME6, MDS3, and KEM3 [66]. Extracellular matrix production is mediated through the expression. of ADH1, GCA1, and GCA2 genes [70].

When the biofilm is established, it allows invasion and cell damage through gene expression from the main histolytic enzymes, secretory aspartic proteases (*SAPs*), and phospholipases (*PLBs*) [71]. The last process is cell dispersion to create new communities that are down-regulated by *UME6* and *SUR7* genes and up-regulated by *PES1* and *NRG1* genes [72]. Changes in expression of these genes during biofilm maturation are controlled by molecule accumulation of *quorum sensing* [70,73]. Genes related to filamentation and adherence are mainly regulated by *EFG1, BCR2,* and *TYE1* transcription factors [74,75].

Cunha JA, et al. evaluated 165 transcriptional regulators in *C. albicans* biofilm, in which 6 mutants were selected for the transcriptional network controls, *bcr1Δ/Δ*, *tec1Δ/Δ*, *efg1Δ/Δ*, *ndt80Δ/Δ*, *rob1Δ/Δ,* and *brg1Δ/Δ* [76]. The authors showed that 1061 genes were regulated by at least one of the 6 biofilm regulators, and they bind to promoting regions of de *BCR1*, *TEC1*,

*EFG1*and *BRG1*. Moreover, they demonstrated that Tec1, Efg1, Ndt80 e Rob1 bind to *ROB1,* Efg1 e Ndt80 bind to *NDT8,* and this regulation is positive. Bcr1, Efg1, Ndt80, Rob1, and Brg1 are activators and repressors of their target genes, whilst Tec1 acts only as a positive regulator. The biofilm network-target genes of these 6 regulators comprehend around 15% of the *C. albicans* genome.

Although *C. albicans* is a model for the study and representation of biofilm formation and its related genes, more studies should be performed with other biofilm-forming microorganisms to understand the adherence and formation mechanisms, for more specific control and treatment.

Conclusions

This study, through a systematic review of high-impact publications and scientific relevance, pointed to the alarming situation of biofilm formation in hospital catheters as a worldwide health problem. Microbial resistance and failure to properly clean and sterilize materials that allow proliferation are among the factors that contribute to biofilm formation in a hospital environment. Virulence factors related to each microorganism are also critical for the gravity of a possible infection in patients.

Biofilm formation in a hospital environment is characterized as one of the main causes of hospital infections, increasing the cost of public health by increasing hospitalization time and possible death. Furthermore, cleaning methods and disinfection comprehend promising alternatives that can destroy the biofilm-forming microorganism, increasing patients' quality of life and minimizing unnecessary expenses from public health.

However, EOs-plant-derived antimicrobial activity has drawn the attention of scientists because it can be used as an alternative to antibiotic resistance from pathogens. As a result, in the last few years, there has been an increase in the growth of several aromatic and medicinal plants due to their efficient antimicrobial properties. In this sense, it is important to provide a review that compiles all the results.

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