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Unveiling the Complex World of Bacterial Biofilms: Implications for Disease and Therapeutic Innovations

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Abstract

Biofilm is a complex formation of microorganisms that adhere to the surface and create a community of different bacterial colonies or a group of single cells that exhibit changes in their physical and genetic appearance and give protection from hostile environments. Biofilm matrix gives bacteria the ability to impede antibiotics, indicating more resistance power and increasing the emersion of infection caused by numerous severely multi-drug resistance bugs. Bacterial biofilms relate to several infectious diseases, including those that affect the auditory, cardiovascular, digestive, reproductive, respiratory, urinary system, etc. Microorganisms including *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Corynebacterium tuberculstearicum*, *Bordetella pertussis*, *Streptococci*, *Salmonella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Clostridium* spp., etc. develop biofilms and are linked to these diseases. This study attempts to evaluate the involvement of bacterial biofilm in numerous diseases affecting various human body systems and reference their diagnostic approach such as PCR, mass spectrometry, FISH, ELISA test, SEM, hysteroscopy, CT scans, DNA microarray, microbiological analyses, etc. Besides, various approaches to control these biofilm-related infections such as natural antimicrobial compounds, nanoparticles, enzyme inhibitors, quorum sensing inhibitors, bacteriophages, DNase therapy, antimicrobial peptides, antimicrobial coatings, liposomes, antibiotics, etc. are also described. The goal of this study is to gain a better understanding of biofilm's involvement with various disease progression along with the different antibiofilm strategies.

Keywords: Biofilm, EPS, antibiotic resistance, diseases, antibiofilm

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1. Introduction

A biofilm refers to a complicated arrangement of microorganisms that can consist of various types of bacterial colonies or single cells grouped together and they adhere to the surface [1]. Generally encased within a self-generated extracellular matrix made of a polymeric substance (EPS) such as protein, lipid, polysaccharides, extracellular DNA, and other elements. They display a modified physical and genetic appearance with respect to their rate of growth and transcription of genes [2,3]. Biofilm formation supports bacteria to develop microbial resistance and shields themselves from adverse environmental stressors, UV radiation, desiccation,

osmolarity, shear forces, disinfectants, nutrient scarcity, host defense, severe pH, high pressure and temperature, high salt concentrations, and traditional antimicrobial agents [4]. Bacteria generate biofilm when planktonic cells are exposed to sub-inhibitory doses of antibiotics, nutritional signals, or cellular identification of precise or non-specific attachment spots on a surface [1]. Bacteria that exist within a biofilm are considerably more resilient to antimicrobial substances compared to those in their individual, planktonic state. Biofilm has the potential to trigger serious infections because it consists of bacteria which is concealed and hibernating in a defensive matrix [2]. Biofilm formation is responsible for 60-80% of microbial infections [3]. In addition, it is capable to persist in the lungs, ears, and teeth causing major illnesses [4]. Antimicrobial resistance against antibiotics is the most concerning matter in biofilm-associated diseases in humans [5].

A variety of biofilm models that vary in the type of surface support for the biofilm and in the spatial distribution of nutrients, oxygen, and water have been used in experiments to simulate the wide range of natural biofilm settings. These are submerged biofilms, which are growing on solid inert surfaces generally found in natural aquatic environments or on catheters. Subaerial biofilms, which frequently consist of cyanobacteria and fungi, grow also on a solid support such as rocks in deserts or on buildings and may give micronutrients, while carbon dioxide, oxygen, and ambient water are provided by the surrounding air. Pellicle biofilms, which receive their nutrition from the liquid phase, flourish at the liquid-air interface and lastly, macro-colony biofilms which develop on nutrient-rich semi-solid agar plates mimic the circumstances under which biofilms grow on decaying organic material like soil or human food materials [6], [7], [8].

2. Biofilms and antibiotic resistance

Bacterial biofilms create a severe threat to world health because of their capability to resist antibiotics, host defense mechanisms, and other external stimuli; which leads to persistent chronic infections [9]. In addition to protecting microorganisms from adverse environmental stressors, biofilms also prevent bacterial colonies from being accessed by antibiotics and the host's immune system. Therefore, the biofilm matrix gives bacteria more resistance power, enabling them to not only withstand adverse conditions but also become resistant to antibiotics, which promotes the emergence of infections caused by harmful bugs such as extensively drug-resistant, multi-drug resistant, and completely drug-resistant bacteria [10], [11]. Even bacteria that do not possess protective mutations or mobile genetic elements carrying resistance genes can become less vulnerable to antibiotics when they are grown in a biofilm state. Bacteria generally regain their susceptibility to antibiotics soon after they are dispersed from a biofilm. In contrast to a genetic change, the quick reversal of resistance following dispersion from a biofilm supports an adaptive resistance mechanism [11], [12].

Biofilm-associated microbial cells exhibit 10 to 1000 fold higher levels of antibiotic resistance than planktonic microbial cells [11]. In comparison to planktonic cells, the horizontal gene transfer rate of bacteria growing in biofilms is higher and a significantly frequent rate of mutation undergoes in biofilms. With the aid of virulence factors and antibiotic resistant genes from resistant to susceptible bacterial species, biofilms enhance the possibility of gene transfer, which results in antibiotic resistance [13], [14]. The quorum-sensing molecules known as autoinducers enable communication between biofilm cells. The modulation of quorum sensing causes a broad alteration in gene expression, boosting virulence and hastening the development of antibiotic resistance [13]. Another cause for antimicrobial resistance, which is defined by relying on bacterial responses to the local environmental conditions, is the differential expression of genes in biofilms. For instance, the rpoS-mediated stress response in *E. coli* biofilm cells may cause physiological changes that contribute to antibiotic resistance [13], [15]. Recent research has discovered genes such as ndvB, tssC1, brlR, rapA, and operons PA1874-1877, PA0756-0757, PA2070, PA5033 that are directly involved in biofilm-specific antibiotic tolerance and resistance [16].

3. Biofilms and infectious diseases

Many persistent infections have been attributed to bacterial biofilms. Four criteria were suggested by Parsek and Singh as signs of medical biofilm infection: 1) the pathogenic bacteria are attached to some exterior/surface or substratum; 2) Infested tissue exhibits bacteria existing in bunches or clusters of cell or micro-colonies enclosed in extracellular matrix; 3) Then the infection is localized, though secondary spreading is feasible; and 4) Finally, antibiotic resistance exists in spite of the susceptibility of the responsible organisms' planktonic cells [17], [18].

4. The auditory system

4.1. Otitis media

In recent clinical studies, a clear link has been established between the existence of a bacterial biofilm located behind the tympanic membrane (TM) and the occurrence of chronic Otitis Media (OM). Inflammation in the middle ear cavity is

one of the major symptoms of OM. Different variations of OM can be distinguished, such as, chronic supportive OM (CSOM), acute OM (AOM), and OM with effusion (OME) [19], [20]. These ailments may result in either momentary or perpetual hearing damage [19]. Around 80% of children will have experienced otitis media by the time they reach the age of 3. Pathogens can cause infection and subsequent disease in the middle ear by entering through the eustachian tube. The anatomical structure of the eustachian tube in the ear is distinguished by its shorter, broader, and more horizontally aligned path, which promotes the transfer of otopathogenic bacteria from the nasopharynx to the middle ear cavity, leading to an increased risk of OM [21]. The most frequently occurring pathogens discovered in preliminary CSOM cases are *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Moreover, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumonia* have been simultaneously detected in polymicrobial biofilms [21], [22]. A study using chinchillas that had no prior middle ear issues demonstrated that intranasal inoculation with *S. pneumoniae* from a patient with recurrent OM resulted in middle ear inflammation, which was detected within 2 days and peaked on the 8th day. This experiment indicated a causal link between nasopharyngeal biofilms and OM [23]. A similar study conducted using a primate model observed the formation of a biofilm when ears were inoculated with *Pseudomonas aeruginosa* [24], [25].

4.1.2. Diagnosis

Doctors typically use a conventional otoscope to visually inspect the eardrum (tympanic membrane) for diagnosing OM [26]. Biofilms are generally thin in nature and can't be easily detected using a standard otoscope [27]. Additional tools such as tympanometry, acoustic reflectometry, and pneumatic otoscopy offer the ability to evaluate the middle ear using functional measurements [28]. A portable diagnostic system integrating LCI with a standard video otoscope successfully detected and measured biofilms in a newly-developed animal model [27]. Culture-independent molecular diagnostic methods, including PCR, mass spectrometry, CLSM, FISH, PnAb, and epithelial cell-specific ELISA, are utilized to investigate the localization and organization of pathogens in their natural surroundings [29].

4.2. The cardiovascular system

4.2.1. Infective endocarditis (IE)

A condition recognized as 'infective endocarditis (IE)' is known to be an infection of the endocardium, which covers the interior surface of the heart and is frequently found on the heart valves or implanted cardiac devices [30]. It is now widely recognized that biofilms are implicated in IE after Costerton et al. identified native valve endocarditis as a biofilm infection brought on by viridans group streptococci [31]. The endothelial periphery of the cardiac valve is initially damaged, followed by the development of a sterile clot-like scar of platelets and fibrin at the site of the lesion. Before microcolonies and a mature biofilm are produced, bacteria first attach to the thrombus, and the mature biofilm fragments can then lead to embolization [17], [18]. Over 80% of cases of IE are caused by enterococci, streptococci, and staphylococci, all of which are known to generate biofilms [32].

4.2.1.1. Diagnosis

A series of clinical, echocardiographic, and microbiologic criteria (Duke criteria) are used to determine the patient's clinical diagnosis of IE [18]. The gold standards for diagnosing IE are blood cultures and valve cultures if a replacement heart valve is required. FISH is used to diagnose endocarditis in some challenging situations involving fastidious or slowly growing organisms such as *Coxiella burnetii*, *Tropheryma whipplei*, or *Bartonella* species. In relation to IE, biofilm is also identified via electron microscopy [18], [19].

4.2.2. Atherosclerosis

Atherosclerosis (AS) is a chronic disorder distinguished by the construction and progression of plaques inside the arteries. Numerous microorganisms have been found in plaques in both epidemiological and microbiologic analysis [33]. Biofilms have a role in the pathogenesis of atherosclerosis, and it has been suggested that the existence of biofilms may increase the chances of plaque rupture, which could be life-threatening [19], [34].

4.2.2.1. Diagnosis

FISH and fluorescence microscopy has been used to detect bacterial biofilms in atherosclerotic arteries. In order to analyze the plaques, a cross-section of a plaque was taken, and the microorganisms present were identified using PCR and sequencing. To further clarify the three-dimensional layout of bacteria within the plaque, these sample were put under the microscope and/or under confocal microscopy [35].

4.3. The digestive system

4.3.1. Sialolithiasis

A condition known as sialolithiasis occurs when calcified masses, also known as sialoliths or salivary stones, develop inside the salivary gland [36]. Due to the discovery of biofilm structures in the centers of the stones in two recent

investigations, it is suggested that the formation of the biofilm may contribute to the etiology of salivary stone generation [37]. In a following study, Kao et al. noted observations of host immune cells, calcium nanoparticles, erythrocytes, and platelets as well as biofilm, using light and scanning electron microscopy [37]. They advanced the idea that biofilm formation results in localized damage, subsequently accompanied by the inflammatory response and calcium deposition. Biofilm development has also been noticed in salivary stones' exterior surfaces [38]. The oral microbiome contains a variety of bacterial species, the majority of which belong to the *Streptococcus* genus [39]. If sialolithiasis left untreated, it can cause the blockage of salivary gland's outflow which can be uncomfortable for the patient and frequent facial swelling during meals [37].

4.3.1.1. Diagnosis

PCR was used to identify a section of the 16s RNA bacterial gene in salivary gland calculi, and electron microscopy was used to confirm the presence of bacteria within the stones. Images resembling gram-positive bacterial cells were seen when gland stone samples were examined under a traditional optic microscope. The same outcomes were seen in scanning electron microscope (SEM) observations. The existence of bacterial DNA was verified by qPCR on the middle slices of the stones [40], [41].

4.3.2. Recalcitrant typhoid fever and predisposition to hepatobiliary cancers

A severe foodborne disease known as typhoid fever is frequently distinguished by abdominal pain, headache, constipation, weakness, and high temperature. Typhoid fever in humans is caused by a bacterial species known as *Salmonella enterica serovar typhi* which expands systematically and generates acute illness by crossing the intestinal epithelial layer, being phagocytosed by macrophages [42]. If it is left untreated, severe side effects like bowel perforation, meningitis, septicemia, intestinal bleeding, and death could occur. According to some evidence, *Salmonella ent erica* subsp. *enterica serovar typhi's* chronic colonization of the gall bladder includes the invasion and attachment of epithelial cells as well as the formation of biofilms on gallstones where the microbes are resistant to bile and antibiotics in large quantities [38]. Bacteria shed from the liver may cause the gallbladder to become infected, either actively (cholecystitis) or chronically (carrier state). There is a fact that the chance of developing gallbladder cancer is 8.47 times higher in *Salmonella* spp. carriers with gallstones [43]. Patients with cholelithiasis (gallstones in the gall bladder) and cholecystitis have had a variety of bacteria found in their gall bladders, including *Salmonella* spp., *Enterococcus* spp., *Bacteroides fragilis, E. coli*, *Acinetobacter* spp., *Proteus* spp., *Citrobacter freundii, Pseudomonas aeruginosa*, *Helicobacter* spp., *Enterobacter* spp., *Klebsiella pneumoniae*, and *Staphylococcus aureus* [44], [45].

4.3.2.1. Diagnosis

Although the recovery of *Salmonella typhi* or *Salmonella* Paratyphi A in bile, gallbladder stones, or tissue of afebrile patients going through elective cholecystectomy is thought to be the gold standard of diagnosis, this method also has little value for public health due to its invasiveness. Detecting organisms in bile or gallbladder specimens has been successful using PCR amplification for *S. typhi*-specific genes (e.g., fimbrial genes staA or fliC), while contemporary PCR approaches have been less successful in finding organisms in feces [46].

4.3.3. Bowel disease

The colon epithelium is shielded from the luminal microbiota in healthy individuals by protective mucosal covering. When this barrier is breached, microbes and epithelial cells come into greater interaction, which can lead to the development of biofilms on the epithelium. These alterations represent a pathological condition that is frequently linked to the emergence of inflammatory bowel disease (IBD). Chronic intestinal inflammation is a hallmark of IBD [(also referred as Crohn's disease (CD) and ulcerative colitis (UC)] [38]. Pathogenic bacterial species such as *Mycobacterium para tuberculosis, E. coli*, *Bacteroides vulgatus*, and *Enterococcus faecalis* are linked to CD and UC, but the majority of the evidence from medical findings and animal models is contradictory [47]. IBD patients were the only ones to have bacteria attached to their colonic epithelium, and *Bacteriodes fragilis* and Enterobacteriaceae controlled these biofilms [38]. IBD-specific mucosal biofilms are thought to trigger pro-inflammatory responses in host tissues via a variety of pathways, according to mounting data [48]. *Enterococcus* spp. and adherent-invasive *E. coli* isolates from Crohn's disease patients are more likely to form a biofilm in intestinal epithelial cell line cultures and on plastic surfaces [49]. Alterations in the abundance of mucolytic commensal bacteria, such as *Akkermansia muciniphila* and Ruminococcaceae, as well as the biogeographic bacterial repositioning that produce glycosidases and proteases, like adherent-invasive *E. coli, Porphyromonas* spp., and *Bacteroides thetaiotaomicron*, may also make it easier for nearby commensal bacteria to access to the intestinal epithelial surface [50].

4.3.3.1. Diagnosis

In the diagnosis and evaluation of intestinal inflammation as well as the interaction between intestinal microbes and IBD pathogenesis, faecal calprotectin and faecal occult blood test (FOBT) are frequently employed [51].

4.3.4. Cancer

One of the most prevalent cancer types in both men and women worldwide is colorectal cancer (CRC) [38]. In the stomach, small intestine, and colon, biofilms have been connected to the beginning and progression of cancer. However, people with CRC had thicker polymicrobial biofilms than healthy individuals as controls, particularly in the ascending right colon [49]. According to Li et al., biofilms can possibly see as an inducer in the adenoma-carcinoma cascade at the beginning of carcinogenesis. Recent research suggests that biofilms have a significant role in colorectal carcinogenesis [38]. Biofilms were discovered in 89% of right sided tumors as opposed to 13% of left sided tumors. Tomkovich et al. found in a recent study that human colon mucosal biofilms in murine CRC-models were carcinogenic regardless of the fact that the microbiota related to the biofilms were from healthy people or CRC patients [38]. *Fusobacterium nucleatum*, genotoxin-producing *E. coli,* enterotoxigenic *Bacteroides fragilis*, and *S. gallolyticus* are strains that are frequently found in mucosa-associated microbiota from CRC patients [49].

4.3.4.1. Diagnosis

By using SEM and FISH, polymicrobial biofilms that adhered directly to the epithelium were found in colorectal cancers [38]. *F. nucleatum*, *B. fragilis*, and *Faecalibacterium prausnitzii* were discovered to be helpful prognostic biomarkers for CRC using a 16S rRNA gene sequencing technique. A promising noninvasive method for generating a distinctive metabolic fingerprint to diagnose or assess the prognosis of CRC is faecal metabolic screening [52].

4.4. Reproductive system

4.4.1. Bacterial vaginosis

In women who are fertile, a significant prevalence of recurrence and relapse characterizes bacterial vaginosis (BV), which is the most prevalent genital tract infection [38]. *Atopobium vaginae*, *Gardnerella vaginalis*, *Megasphaera* spp., and other anaerobic bacteria, along with a reduction in the amount of protective lactobacilli, are typical anaerobic bacteria found in BV [38]. Without a doubt, the presence of a thick, multispecies vaginal biofilm is linked to BV, with *G. vaginalis* being the dominant species (up to 90%) [53]. The vaginal epithelium is most likely originally colonized by *G. vaginalis*, which leaves a foundation for subsequent species to cling on [38]. Utilizing a new multiplex peptide nucleic acid fluorescence in situ hybridization technique, it was recently demonstrated that *G. vaginalis* was capable of adhering to and displacing defensive lactobacilli from vaginal epithelial cells, while other BV-associated anaerobes such as *P. bivia, M. mulieris, A. vaginae,* and *F. nucleatum* were less virulent [54]. All of the BV-associated anaerobes that were tested had the ability to promote the growth of *G. vaginalis*, however, this effect was more protuberant when *M. mulieris* and *P. bivia* were present [55].

4.4.1.1. Diagnosis

The direct probe assay, also known as the BD AffirmTM VPIII probe assay, is a molecular method for the diagnosis of BV. The fluorescence in situ hybridization method has also been evaluated for bacterial vaginosis, and it has shown to be more specific than other conventional techniques for identifying bacteria that form biofilms in vaginal samples [56].

4.4.2. Chronic endometritis

Chronic Endometritis (CE) is defined by the ongoing endometrial mucosal irritation that is brought on by common bacteria colonizing the uterus, specifically *Pseudomonas aeruginosa, Enterococcus faecalis*, *Proteus* spp., *Gardnerella vaginalis*, *Streptococcus* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus* spp., genital pathogens as *Ureaplasma* spp. and *Mycoplasma hominis* and *Neisseria gonorrhoeae, Chlamydia trachomatis*, *Ureaplasma urealyticum* and yeasts like *Candida* spp., *Saccharomyces cerevisiae* [57]. CE may be asymptomatic or followed by minor symptoms such as pelvic pain, abnormal uterine bleeding (AUB), dyspareunia, and vaginal discharge [58]. The most prevalent bacteria were discovered to be *Lactobacillus* spp., followed by *Atopobium* spp., *Gardnerella* spp., *Sneathia* spp., and *Prevotella* spp. [38], [57].

4.4.2.1. Diagnosis

The current CE diagnosis relies on the histopathological detection of plasma cells in the stromal compartment following office endometrial sample using conventional staining and/or immunohistochemistry for CD138. Hysteroscopy has been suggested by several authors as a trustworthy alternative to the gold standard diagnostic for CE [59].

4.4.3. Mastitis

The majority of cases of mastitis, an inflammatory disease of the mammary gland that mostly occur at the time of lactation. Infectious mastitis is thought to be primarily caused by *S. aureus*, although *E. coli*, streptococci and coagulase negative staphylococci (CNS) may also be present. *S. agalactiae* (which causes infectious mastitis), CNS, *S. dysgalactiae*, *Klebsiella* spp., *Citrobacter* spp, *S. uberis*, *E. coli*, Enterococci, and *Enterobacter* spp., are additional bacteria linked to mastitis

[38], [60]. However, *in vivo* investigation by Hensen et al. using microscopic examination of *S. aureus* in mammary tissue did reveal the presence of biofilm. It has been demonstrated that lactose and milk both promote the *in vitro* production of a *S. aureus* biofilm [38]. *Staphylococcus aureus* can attach to and colonize the mammary gland epithelium more easily when biofilms are formed [61].

4.4.3.1. Diagnosis

Bacteriological tests, clinical examination, cytological examination of milk (indirectly using the California Mastitis test or the Whiteside test, direct using fluoro-optoelectronic counters and microscopic cell counting), measurement of milk electrical conductivity, and imaging techniques (ultrasonography, endoscopy, infrared thermography) are all used as mastitis diagnostic procedures [62]. A common biomarker for diagnosing bovine mastitis is haptoglobin (acute phase protein) [63].

4.4.4. Pelvic inflammatory disease

Pathogenic bacteria enter the uterus and induce endometritis and pelvic inflammatory disease (PID). STIs or BV-related pathogens are responsible for more than 85% of PID cases [64]. Less than half of those instances are attributable to *N. gonorrhoeae* or *C. trachomatis*, indicating a significant role for non-BV-related pathogens (such as *Mycoplasma genitalium*) and BV-associated anaerobic bacteria in the pathophysiology of PID and endometritis [64].

4.4.4.1. Diagnosis

The Centers for Disease Control and Prevention (CDC) guidelines serve as the foundation for the clinical diagnosis of PID. Laboratory data or imaging investigations are typically not required for PID because it is a clinical diagnosis, but they might be useful in making the diagnosis or determining its severity. Additionally, PID can be directly treated using ultrasonography as a diagnostic tool [65].

4.5. Integumentary disease

4.5.1. Wound infections

Microorganism-filled wounds typically heal well, however, occasionally, bacteria, in particular, grow, hinder healing, and damage wound tissues, resulting in an infection [38]. The majority of anaerobic species found in human and animal wounds include *Peptostreptococcus* spp., *Bacteroides* spp., *Clostridium* spp., and *Fusobacterium* spp. [66]. While *Staphylococcus aureus* is the most frequently isolated bacterial species, chronic wounds can be colonized by a variety of diverse bacterial species. Aerobic bacteria are frequently detected on the surface of chronic wounds, such as *Pseudomonas aeruginosa*, *S. aureus*, and *S. epidermidis*, although anaerobic species prevail in deeper tissue [38]. Recently, it has been discovered that bacteria from chronic wounds live in biofilms and contribute to infection and slowed recovery [67].

4.5.1.1. Diagnosis

The level of examination for biofilm diagnostic techniques can be broadly divided into morphological assay, microbiology assay, and molecular assay [68]. SEM and confocal laser scanning microscopy are two of the most trustworthy forms of diagnostic methods, according to the European Society of Clinical Microbiology and Infectious Diseases study group for biofilms. New techniques have been developed, such as PCR, FISH, and denaturing gradient gel electrophoresis, to increase the precision and scientific nature of the clinical diagnosis [69].

4.6. The respiratory system

4.6.1. Rhinosinusitis

Rhinosinusitis (RS) is a disease that is caused by nasal and paranasal sinuses inflammation and microbial dysbiosis [38]. The disease is mainly characterized by obstruction, superantigen stimulation of the allergy, nasal blockage, nasal discharge, or fullness, ciliary disorders, fungal or bacterial infection, immune system, and immune insufficiency [23]. Patients with chronic rhinosinusitis (CRS) who have biofilms in their sinus mucosa have reduced mucociliary clearance and inadequate sinus outflow. In 2004, with the help of electron microscopy, the first existence of biofilm was found in a CRS patient [70]. The biofilms of CRS patients is enriched with *Corynebacterium tuberculstearicum*, *Cutibacterium acnes*, *Staphylococcus aureus*, *Haemophilus influenza*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, and *Moraxella catarrhalis* in sinuses [70].

4.6.1.1. Diagnosis

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), fluorescence in situ hybridization (FISH), and confocal laser scanning microscopy (CLSM) are a few techniques for detecting biofilms in RS. The best method for detecting biofilms in CRS is now CLSM [71].

4.6.2. Pharyngitis and laryngitis

Pharyngitis or sore throat is defined by recurrent bacterial infections of lymphoid structures such as tonsils and adenoids which may lead to hypertrophy of adenoid or tonsillar tissue. The symptoms which may be observed during this condition are harsh squawking sounds, adenoids or tonsillar hypertrophy, cervical adenopathies, apnea and all of these indicates the presence of biofilms in tonsils [38]. It had been found that biofilms in adenoid tissue may act as a reservoir for infection in other parts of the respiratory tract [38]. In a number of studies, after performing adeno- and tonsillectomy biofilms had been detected. The bacterial species responsible for this condition are *Streptococcus* spp., *S. aureus*, and *Haemophilus* spp. [29], [38]. *In vitro* experiments, it was shown that epithelial cells are protected by *S. salivarius* and *S. oralis* biofilms from internalization, cytotoxic effects, and GAS adherence. Comparing patients with obstruction to those with chronically irritated adenoids and tonsils, Al-Mazrou and Al-Khattaf noticed that the prevalence of biofilm was noticeably higher in the latter group [38]. Biofilm was found in 62% of individuals with chronic laryngitis after true vocal fold samples were examined using PCR and confocal laser scanning microscopy [38], [72].

4.6.2.1. Diagnosis

Previous studies have shown the value of MtP, CRA, confocal laser scanning microscopy, and/or PCR techniques for the identification of crucial virulence components in pharyngitis, specifically the capacity for biofilm formation in *Staphylococcus* species [73]. A biopsy specimen or multiple were collected for histopathological analysis of laryngitis. CLSM used BacLight LiveDead dye (Molecular Probes) to look for signs of bacterial growth in one biofilm specimen and PCR with a microarray-based diagnostic assay to look for a specific microbiological diagnosis in laryngitis [74].

4.6.3. Cystic fibrosis

The genetic disorder Cystic Fibrosis (CF), which is autosomal recessive in Caucasians, was the first infection for which the role of biofilm in pathogenesis was established, and leads to chronic infections and viscid mucus production, particularly affecting the respiratory and digestive systems [21], [38], [75]. Many polymorphonuclear leukocytes (PMNs) infiltrate the area in reaction to the biofilm's presence, causing chronic inflammation that leads to tissue destruction, bronchiectasis, loss of lung function, and airway blockage [38]. CF-promoting gram-negative bacteria are *Achromobacter xylosoxidans*, *Burkholderia cepacian* complex, *Haemophilus influenza*, *P. aeruginosa*, *Stenotrophomonas maltophilia*, and gram-positive bacteria includes Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus* [21], [76]. Evidence of a chronic *P. aeruginosa* biofilm has been found in lung tissue, sputum, and lung abscesses [38]. Patients with cystic fibrosis have viscous secretions in their lungs, which promote an anaerobic environment that encourages *P. aeruginosa* to change from free-floating planktonic bacteria to biofilm. Airway epithelial cells release more iron into the extracellular space as a result of the CFTR gene mutation, which encourages *P. aeruginosa* biofilm development [23], [77].

4.6.3.1. Diagnosis

Traditionally, bacteria are detected through culture on agar plates using samples of sputum, cough (or oropharyngeal) swabs, or bronchoalveolar lavage [78].

4.6.4. Non-cystic fibrosis bronchiectasis

Non-Cystic Fibrosis Bronchiectasis (NCFBE) is a progressive disorder of the respiratory system that is caused by chronic inflammation, mainly specified by irreversible bronchi dilation, with signs including chronic cough, abnormal enlargement of one or more airways, high sputum creation, and frequent infectious exacerbations [75]. NCFBE is frequently brought on by a preliminary lesion (such as a bacterial infection of the bronchi), which is then followed by inflammation produced by an overreactive innate immune response. The predominantly observed bacterial species responsible for bronchiectasis in the lower respiratory tract are *P. aeruginosa*, *S.pneumoniae*, *S. aureus*, *Prevotella* spp., *M. catarrhalis and Veillonella* spp. [21], [75].

4.6.4.1. Diagnosis

High resolution chest CT scans are the primary diagnostic tool for bronchiectasis [79]. High resolution computer tomography (HRCT) is used in radiology to make the diagnosis. Lack of bronchial tapering and bronchial dilatation are two HRCT diagnostic criteria for non-CF bronchiectasis [80].

4.6.5. Chronic obstructive pulmonary disease

Chronic Obstructive Pulmonary Disease (COPD) is a dynamic disorder of parenchyma of the lungs and respiratory system. Normal breathing is hampered by COPD's non-completely reversible airflow restriction. The lung parenchyma, pulmonary vasculature, peripheral respiratory tract, and central respiratory tract are only a few of the tissues whose structural changes are brought on by innate immune cell activation triggered by respiratory tract inflammation in COPD patients. Chronic cough, excessive mucus production, mucociliary dysfunction, and dyspnea are among the symptoms of COPD in patients. Nontypeable *Haemophilus influenzae* (NTHi), *M. catarrhalis*, *S. pneumonia*, and *P. aeruginosa* are common

COPD pathogens. Sialic acid's sialylation of lipooligosaccharides (LOS) supports the growth of NTHi biofilms. Enterobacteriaceae and *H. parainfluenzae* are two more gram-negative bacterial species that are seen in COPD patients [21], [75], [81].

4.6.5.1. Diagnosis

The clinical microbiology laboratory uses the demanding growth requirements of hemin and NAD to identify *H. influenzae*. *Haemophilus* species are typically isolated from clinical samples using chocolate agar, which is incubated at 33–37 °C and 5–10% CO_2 [82].

4.6.6. Ventilator-associated pneumonia (VAP)-mediated chronic infection

Within hours of endotracheal intubation, endotracheal tubes surface rapidly produce biofilms, and bacteria begin to colonize them. Patients receiving mechanical ventilation (MV) in intensive care units (ICU) are at risk for developing nosocomial pulmonary infections [75]. They are frequently put in a setting with resident flora and a coating of mucus, which may facilitate the transition of harmful bacteria adhered to the patient's endotracheal tubes surface. According to reports, 70% of patients with VAPs had endotracheal tube biofilm interactions with their lungs [75], [83]. *Prevotella and Streptococcus* species are among the most prevalent members of the oral flora, however ESKAPE microorganisms (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp.) are also present. Endotracheal tube biofilms usually contain *Porphyromonas gingivalis*, the most frequently found Gram-negative bacterium, as well as *S. mutans* and *S. epidermidis*, two separate types of facultative anaerobic, Gram-positive bacteria [75], [84].

4.6.6.1. Diagnosis

Clinical evaluation combined with microscope examination and culture of tracheal secretions is one of the diagnostic methods for VAP-mediated chronic infection. Three factors are typically used to make the diagnosis of VAP- systemic infection symptoms, new or deteriorating infiltrates on the chest roentgenogram, and bacteriologic proof of pulmonary parenchymal infection. However, the use of a microscope to examine tracheal aspirates may be useful in making a diagnosis of VAP in some patients. Microbiologic diagnosis of VAP using nonbronchoscopic and bronchoscopic techniques are also used [85].

4.7. The urinary system

4.7.1. Bacterial prostatitis

Bacterial prostatitis (BP) is characterized as an infection of the urinary system that affects the genitalia and pelvis, as well as bacterial prevalence in expressed prostatic secretions. Urosepsis, bladder infections, reduced fertility, prostatic abscesses, and even mortality are potential consequences of BP. In chronic bacterial prostatitis, the intraprostatic ductal reflux and urethral flow patterns aid in the formation of biofilm as the bacteria cling to the uroepithelium and enter the prostate from the urethra [38], [86]. *E. coli*, *Klebsiella spp*., *P. aeruginosa*, *Serratia spp*., *Proteus mirabilis*, and other Enterobacteriaceae, moreover *E. faecalis*, are the species most frequently linked to BP [87].

4.7.1.1. Diagnosis

Urinalysis, urine culture, prostate testing, a urine voiding examination, and a history are performed as part of the initial evaluation of chronic BP [88]. Quantitative successive bacteriological localization cultures are also used for the diagnosis of CBP. The first-voided urine (VB1), midstream urine (VB2), post-prostate massage urine (VB3), post-prostate massage urine (VB3), and expressed prostatic secretions (EPS) are all sampled during the Meares-Stamey four-glass test, which is regarded as the gold standard for the diagnosis of CBP. In clinical practice, a simple two-glass test is frequently used for diagnosis because of its simplicity and convenience compared to the four-glass test, which is more complicated [88], [89]. In order to make the evaluation of BP in clinical practice simpler, the accurate novel enhanced diagnostic technique for semen culture has been adopted as an alternative standard procedure [90].

4.7.2. Urinary tract infections

Infections of the urinary tract (UTIs) are exceedingly common in people and develop when bacteria, typically from the perineum and rectum, pass through the urethra and colonize in the kidneys, ureters, urothelium, urethra, and bladder [19]. Clinical classification of UTIs has been done based on clinical features and how they affect morbidity and treatment. Some conditions include asymptomatic bacteriuria, severe UTI, UTI associated with indwelling catheters, acute uncomplicated pyelonephritis in young women, acute uncomplicated cystitis in young women, and recurrent cystitis in young women [86]. The presence of dysuria, urine frequency, urgency, and/or lower abdomen pain are among the symptoms, which vary depending on where the infection is located anatomically. An increasing amount of evidence points to the role of ongoing bladder infection brought on by intracellular bacterial communities (IBC) [38]. *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Citrobacter* spp., *Morganella* spp., *Acinetobacter* spp., *Enterobacter* spp., group B

Streptococcus, *Serratia* spp., *Candida* spp., *Staphylococcus saprophyticus*, *Providencia* spp., and *Staphylococcus aureus* are the bacterial species involved in UTI [87]. Uropathogenic *Escherichia coli* (UPEC) is prevalently responsible as a common disease-causing organism for both complicated and uncomplicated UTI [91].

4.7.2.1. Diagnosis

The Congo Red Agar (CRA), microtiter plate, and tissue culture plate (TCP) methods can be employed for the study of biofilm in UTIs [92]. Simple tube methods and spectrophotometer assays can both be used for biofilm detection and quantitative estimation, respectively [93].

4.7.3. Catheter-associated UTI

Catheter-associated urinary tract infections (CAUTIs) are UTIs that occur in patients who have an indwelling urinary catheter in place at the time of the infection or within 48 hours of it beginning [94]. Bacterial species responsible for catheter-associated urinary tract infections are *Pseudomonas aeruginosa*, *P. mirabilis*, *E. coli*, *Klebsiella, enterococci*, *Enterobacter,* or *Klebsiella* spp. In addition, *Proteus vulgaris* and *Providencia rettgeri* can generate crystalline biofilm [86], [91]. *Proteus mirabilis*, which induces CAUTIs, relies on the expression of mannose-resistant Proteus-like (MR/P) pili for adhesion and the establishment of biofilms on the catheter and in the bladder [87], [91].

Recurrent urinary tract infections (UTIs), a defining characteristic of chronic bacterial prostatitis, may occur from uropathogens' increased ability to cause acute prostatitis and persist in the prostatic secretory system as a result of the development of biofilms [87]. Observations made using these experimental models have demonstrated that bacteria build dense, coherent biofilms that are connected to contaminated drainage spouts, extend proximally into the drainage bag, and eventually enter the catheter [95]. The rising bacterial biofilm seems to be driven by two distinct mechanisms in the absence of antibiotics: first, within the glycocalyx substance of the biofilm, extremely pathogenic bacteria cells had spread across the catheter's surface, and second, rapidly ascending planktonic bacteria cells in the urine, possibly helped by turbulence, an outcome of urine flowing over the biofilm front [95], [96].

4.7.3.1. Diagnosis

Tissue culture analysis (TCA), the industry-recognized gold standard approach, was used to find biofilm in CAUTIs [97]. The importance of candiduria in catheterized patients and the use of microbiological catheterization and biofilm identification by tube methods to direct treatment regimen [98]. Blood agar, MacConkey's agar, or eosin-methylene blue agar (which indicates lactose fermentation) are all culture-based methods for laboratory diagnosis of CAUTIs that can be used with urine or catheter biofilms [99].

4.7.4. Biofilms on JJ stents

Reid was the first to describe the formation of biofilm on ureteric stents and found that 90% of indwelling silicone JJ stents had adherent microorganisms colonizing them. Clinically, the accumulation of encrustation and infection on indwelling stents is associated with discomfort, haematuria, obstruction, and sepsis. These stents get encrusted with struvite and calcium phosphate as a result of urea hydrolysis, an elevation in urine pH, and the development of biofilm, especially urease-producing bacteria [100], [101].

4.7.4.1. Diagnosis

Microorganisms in biofilms of JJ stents are diagnosed by using the scanning electron microscope, tissue culture plate assay method, Quantitative PCR technique, X-ray Photoelectron Spectroscopy (XPS), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), microbiological analyses etc. [102].

4.8. The skeletal system

4.8.1. Prosthetic joint infection

One of the most concerning side effects of arthroplasty surgery is prosthetic joint infection (PJI), which is so dangerous because it is resistant to treatment with current medications [103]. The pathophysiology of prosthetic joint infection involves the development of biofilms inherently [104]. *Staphylococcal* species that are Gram-positive, such as *Staphylococcus aureus*, and coagulase-negative, such as *S. epidermidis*, are the most frequent microbes that cause prosthetic joint infection [103]. According to the timing of the development of symptoms after implantation, PJIs divided into: (1) early infection, usually brought on by extremely aggressive germs like *Staphylococcus aureus* or Gram-negative bacilli (like *E. coli*), occurs during the first three months following surgery, (2) delayed infection, generally brought on by coagulase-negative staphylococci or *Priopionibacterium acnes*, which are less aggressive bacteria; this infection typically occurs 3–24 months after surgery, and (3) late infection, lasting more than 24 months, frequently brought on by aggressive bacteria including *Staphylococcus aureus*, and Gram-negative bacilli [104].

4.8.1.1. Diagnosis

The quantitative variant (qPCR) has been utilized most frequently in research using PCR (Polymerase Chain Reaction) for PJI diagnosis. Even if the bacteria are contained within biofilms, FISH can detect them in illnesses that are culture-negative, such as PJI. PhyloChip and HuGChip, two existing microarrays that extensively represent the human microbiome and may be used to identify PJI, as well as custom microarrays that include pathogens frequently linked to PJI, are also available [105].

5. Approaches to control the biofilm-related infection

5.1. Natural antimicrobial compounds

Ibicella lutea, *Coccinia grandis* extracts, vanilla, patchouli, volatile oils from *Salvia officinalis* and *Rosmarinus officinalis*, ylang-ylang, *Mentha piperita*, *Satureja hortensis, Eugenia caryophyllata*, and lichens are just a few examples of the many molecules with an antibiofilm activity that plants can produce. Manuka honey and probiotic compounds also exhibit antimicrobial activity against biofilm [87], [106]. As a natural nutritional supplement, arginine has demonstrated excellent efficacy against the development of biofilms, pathogenicity, coaggregation, and bacterial proliferation. Oral pathogens such as *C. albicans*, *P. gingivalis*, *A. odontolyticus*, *F. nucleatum*, *Lactobacillus acidophilus*, *A. actinomycetemcomitans*, *E. faecalis*, and *A. naeslundii* can be inhibited by the arginine-rich polycationic protein protamine. L-arginine has been demonstrated to prevent *Prevotella oris* and *P. gingivalis* from aggregating together [107], [108].

Green tea's EGCg (epigallocatechin gallate) was a potent antibacterial agent against the biofilms of planktonic *E. faecalis*, *P. gingivalis*, and *F. nucleatum*. It can also be combined with antimycotics to combat the biofilm of *C. albicans*. *S. mutans* growth and adhesion to surfaces have been inhibited by ethanolic extracts of propolis (EEPs) [109]. *Salmonella* spp., *Staphylococcus aureus*, *Helicobacter pylori*, and *Escherichia coli* are just a few of the pathogens that cranberries have demonstrated antibacterial action against [109]. The most effective elements in cranberries that can prevent *S. mutans* from forming biofilms are proanthocyanins (PACs) and flavonols. A-type PAC inhibits *P. gingivalis*'s ability to build biofilms, adhere to human epithelial cells, invade those cells, and engage in proteinase activity. Antioxidants like N-acetylcysteine and natural items like tea have all been found to reduce the adherence of bacteria and the development of biofilms [108], [109], [110].

5.2. Nanoparticle

Depending on the nanoparticles' form, size, concentration, length of contact with the surface, and other physicochemical parameters of the environment, metal cations and associated compounds exhibit significant antibacterial properties. Nanoparticles can damage cellular membranes, enter bacterial cells, and attach to chromosomal DNA because of their small size [94], [111]. The extraordinary antibacterial properties of the nanostructured metallic ions and their compounds may hold the key to the effective prevention and treatment of hazardous biofilms in the ecological, industrial, and medical fields [87]. Metallic (copper, gold, iron, silver) and polymer (poly(L-lactic acid), chitosan, poly(caprolactone), and poly(lactic-co-glycolic acid) nanoparticles, as well as silica nanoparticles, graphene, and quaternary ammonium polyethyleneimine have all been reported to be active at improving biofilm treatment [75], [109].

The most effective silver compounds against oral biofilm infections are silver nitrate and silver nanoparticles (AgNPs). When used against *Enterococcus faecalis*, AgNPs demonstrate their antibiofilm capability. *Streptococcus mutans*, other streptococci, and *E. faecalis* were inhibited by chitosan in terms of growth and adhesion. Ag-conjugated CNPs can lessen the development of biofilm on dental implants by preventing *S. mutans* and *Porphyromonas gingivalis* from growing and adhering to surfaces. MSNs encapsulated CHX (CHX@MSN) and Mesoporous silica nanoparticles (MSNs) have exhibited significant antibacterial activity against *Aggregatibacter actinomycetemcomitans*, *E. faecalis*, *Fusobacterium nucleatum*, *S. mutans*, and *S. sobrinus* in monospecies biofilm. Strong antibacterial and antibiofilm actions have been demonstrated by the QAS, 12-methacryloyloxydodecyl-pyridiniumbromide (MDPB) against *Prevotella nigrescens*, *F. nucleatum*, *S. mutans*, and *E. faecalis* [108], [112], [113].

5.3. Enzyme inhibitors

Crystalline biofilms and encrustation on catheters are frequently observed when urease-producing bacteria are present. In order to reduce the related encrustation, the pH of *P. mirabilis* was raised *in vitro* using urease inhibitors to limit urea degradation [87], [94]**.** Bilberry extracts with anti-adhesin properties, diguanilat cyclase inhibitors, and low surface acoustic waves, plant extracts such as fluorofamide, prune juice, vanillic acid, lactones, are examples of urease inhibitors [87]. LpxC, an enzyme necessary for the formation of lipid A (the anchor membrane for LPS), and inhibitors that bind to it are regarded as recently created antibacterial compounds [75].

5.4. Matrix-targeting enzymes

The *P. aeruginosa* glycosyl hydrolases PelA and PslG disrupt bacterial biofilms and make the bacterium more susceptible to antibiotics and neutrophils. The activity of fluconazole is increased *in vivo* by ß-1,3-glucanase, which targets the ß-1,3-glucan-rich EPS of *C. albicans*. *In vitro*, adherent *A. fumigatus* biofilm production is inhibited by Sph3, a glycoside hydrolase that breaks down GAG, and pre-formed fungal biofilms are ruptured [84], [114]. The lyase cellulase, which can break down cellulose, has been shown to be efficient against Bcc biofilm and *P. aeruginosa*. Certain bacteria can be effectively treated using matrix-targeting enzymes. Examples include Thermonuclease (Nuc) for MRSA, Dispersin B for Bcc, *S. aureus*, and *S. epidermidis*, Alginate Lyase for *P. aeruginosa*, *A. fumigatus*, and *A. xylosoxidans*, Subtilisin for *A. xylosoxidans*, Proteinase K on NTHi, Lyticase on *C. albicans*, and Serratiopeptidase on *S. aureus* [115], [116].

5.5. Quorum sensing inhibitors

Inhibitors of quorum sensing (QS) may be useful for controlling biofilm related to UTIs. *In vitro*, azithromycin inhibited QS-dependent behaviors, whereas animal studies have shown that furanones can inhibit the development of *S. epidermidis* biofilms on urinary catheters [87], [94]. Current research has shown that several natural substances can modulate QS phenotypes to exhibit antibacterial capabilities [87]. Inhibitors of cyclic-di-GMP, quorum sensing signaling and (p)ppGpp demonstrate potential anti-biofilm activity *in vitro* in a wide range of bacterial species and may result in innovative therapeutic approaches to stop the production of biofilms. For *P. aeruginosa*, macrolides efficiently disrupt quorum sensing [23], [84]. Medications including azithromycin, ceftazidime, and ciprofloxacin inhibited *P. aeruginosa's* ability of quorum sensing [23], [117].

Along with *P. aeruginosa*, RNA-III inhibiting peptide (YSPWTNF-NH2, RIP) can stop the formation of a QS system found in *S. aureus* biofilms [75]. Quorum-sensing inhibitors (such as clarithromycin, furanone, and NVC-422 as well as azithromycin at subinhibitory concentrations) have also been investigated as a potential treatment for otolaryngological infections caused by biofilms [106]. Erythromycin, clarithromycin, and roxithromycin all diminish the levels of the autoinducers C4-HSL and 3-O-C12-HSL. By using autoinducer analogs to stop transcription activator R-protein activity, quorum sensing may be impaired [117], [118].

5.6. Bacteriophages

Bacteriophages, often known as phages, are bacterial viruses that have the ability to cause the host bacteria to lyse in a therapeutic manner. Several studies have demonstrated their potential use against a variety of strains that produce biofilms, such as *S. aureus, P. aeruginosa,* MRSA, Bcc, and *C. albicans* [119]. Bacteriophages are a different antibacterial strategy for inhibiting catheter growth linked to biofilms. Catheters can be coated with T4 bacteriophages, which are effective against *E. coli*, and coli-proteus bacteriophages, which are effective against proteus. When catheters were treated with phage, it was shown that biofilm formation was reduced by almost 90% as compared to control catheters [87], [94]. T4 phage lowered the MBEC of cefotaxime by 2 and 8 times, respectively, against *E. coli* biofilms. SAP-26 phage by itself was able to eradicate about 28% of the bacteria in the biofilm after 24 hours of treatment. Using a combination of RNA phages, biofilm formation was slowed by 94%, and existing biofilms were eliminated by 88%. Over 48 hours of K and DRA88 phage treatment, a significant decrease in biofilm biomass was seen in each case. When LexA3-producing phage and ofloxacin were used together *in vitro*, the amount of biofilm cells that were destroyed was enhanced by around 1.5 and 2 orders of magnitude in comparison to unmodified phage plus ofloxacin and no phage plus ofloxacin, respectively. The AT7-engineered phage was about 2 orders of magnitude more successful than the non-enzymatic wild-type phage at reducing *E. coli* biofilm cell counts after 24 hours of treatment, resulting in 4.5 orders of magnitude drop in cell counts [120], [121], [122].

5.7. DNase therapy

A few examples of microbial species that have the ability to form biofilms and utilize DNA as an essential component of the matrix include *A. fumigatus*, *S. aureus*, *C. albicans*, and *P. aeruginosa* [84]. *In vitro* biofilm, antifungal activity is increased when extracellular DNA is broken down by recombinant DNase, which also impairs the structural integrity of the biofilm [84]. It is common practice to treat cystic fibrosis lung disease with inhaled recombinant DNase I (dornase alfa), however, this is mostly because of its mucolytic abilities [84]. With the addition of DNase treatment, biofilms produced by various species linked to chronic pulmonary infections, such as *Klebsiella pneumoniae*, *H. influenzae*, *C. albicans*, *S. aureus*, *P. aeruginosa*, Bcc, NTHi, NTM, *A. fumigatus*, and *S. pneumoniae* are all discovered to be susceptible to antimicrobial agents [75].

5.8. Antimicrobial peptides (AMPs)

One type of viable-length oligopeptide is called an AMP (from five to over a hundred amino acids) [75]. Cathelicidins of animal and human origin are the most common naturally occurring AMPs that can inhibit the formation of biofilms which function by activating cathelicidin activity and rupturing cellular membranes of organisms [75]. Non-cathelicidins

of both animal and human origin, such as pleurocidins, defensins, histatins, lactoferrin, and defensin-like peptides, constitute the naturally occurring anti-biofilm AMP group. AMPs of bacterial origin with anti-biofilm capabilities include class I bacteriocins with post-ribosomal modification (nisin), basically polymyxins (polymyxin B or colistin) and unmodified class II bacteriocins (pediocin PD-1). The histatin analogue in synthetic AMPs, dhvar4, displayed improved antibiofilm activity against *S. mutans* and *C. albicans*. Another category of artificial AMPs, such as (RW)n-NH2 based AMPs and (RW)den 4D drimers, are created using repetitions of the amino acid tryptophan (Trp or W) and arginine (Arg or R). Inhibiting biofilm development by 93.5% at 40 M, they demonstrated an impressive biofilm eradication effect [75], [123]. Magainin, nisin and Human β-defensin 1-3 are examples of natural AMPs, while histatins 5 and 8, B-17, and human neutrophil peptides 1 and 2 are examples of synthetic AMPs that promote intracellular targeting, metabolic inhibition mechanisms (inhibition of microbial functional proteins, DNA and RNA synthesis) and transmembrane pore creation [108], [109].

5.9. Non-antibiotic treatment

Another hypothesis for treating CF biofilms without using antibiotics is sodium nitrite [23], [124]. Another cutting-edge treatment for preventing biofilm formation is iron limitation. Deferoxamine and deferasirox, two iron chelation medicines, work well with tobramycin to treat existing *P. aeruginosa* biofilms and stop the growth of new biofilms on CF airway cells [23], [125]. Many NO (nitric oxide) sources can reduce the quantity of biofilm respiratory infections including *S. aureus*, *P. aeruginosa*, and *S. epidermidis* and form as well as have direct antibacterial effects because of intricate reactions between the NO radical superoxide, metals, and thiol. Moreover, it has been discovered that NO inhibits the development of *C. albicans* biofilm [94], [126]. Thiolanox is a commercially available NO medication that uses NO gas inhalation treatment to treat CF patients' lung infections and enhance their lung function [75]. In both *in vivo* and *in vitro* biofilm removal with Ga(NO3)3 was successful, demonstrating that this compound used a special method to kill the bacteria that form biofilms [127].

By preventing the development of new biofilms and eradicating bacteria from existing biofilms, CORM-2 ([Ru(CO)3Cl2]2) is able to successfully weaken *P. aeruginosa* biofilms [75]. Lead substances with high affinity and specificity for the GtfC catalytic domain have demonstrated potential for regulating oral biofilm [109]. A distinctive class of structural analogs of diazaspiro-decane that were particularly effective against *C. albicans*. The planktonic cell development is unaffected by these compounds, which inhibit *C. albicans* filamentation and biofilm formation. Even at relatively low concentrations, silver compounds can inhibit the formation of bacterial biofilm. Nitric oxide (NO) donors disrupt bacterial signaling and cause oxidative stressors and cellular nitrosative. By the suppression of the enoyl acyl carrier protein reductase enzyme, triclosan prevents bacterial fatty acid production. AgNPs alter the permeability and function of cellular membranes as well as cause oxidative stressors, disable bacterial enzymes by attaching to thiol groups, and generate oxidative stress [108], [128], [129]

5.10. Antimicrobial coatings

To develop novel biomaterials that are more resistant to microbial colonization or to serve as carriers or delivery systems for antimicrobial medications, nanotechnology is being used. Various antimicrobial substances, such as antibiotics, were employed to coat and impregnate urinary catheters (nitrofurazone, ciprofloxacin, norfloxacin, and gentamicin). Artificial cationic peptides, silver, and bacteria expressing a biofilm-degrading enzyme are involved in biofilm degradation. Hydrogels, nanoparticles MgF fluoride, ytrium (YF3), CaO, nitrous oxide, nitrofurazone (nitrofuran), violet gentian with chlorhexidine, antagonistic nonpathogenic microorganisms, and minocycline plus rifampicin are also involved in the antibiofilm activity [94], [126]. The tympanostomy tube's albumin coating may help slow the establishment of biofilms by reducing the adhesion of foreign objects by inhibiting the binding of fibronectin [23].

5.11. Liposomes

Hydrophilic and hydrophobic compounds can both be carried by liposomes to their target region for delivery. In addition to shielding the medications from the environment, this serves to extend their half-life. To test its antibiofilm activity against *E. coli*-induced CAUTI, ciprofloxacin-containing liposomes embedded in hydrogel-coated catheters were tested in a rabbit model. In contrast to the control group, those who received liposomal ciprofloxacin exhibited a delayed development of positive urine cultures [94], [126].

5.12. Antibiotics

Different antibiotic classes have different success rates in penetrating the biofilm matrix. Compared to fluoroquinolones, rifampicin, and macrolides, for example glycopeptides and beta-lactams diffuse more slowly, whereas cationic aminoglycosides are contained by the negatively charged polymers of the biofilm matrix. Clarithromycin plus

vancomycin and roxithromycin plus imipenem are the two most effective antibiofilm combinations mentioned in the literature [87], [108]. Moreover, it was discovered that clarithromycin reduced CF *P. aeruginosa* biofilms more successfully and synergistically than tobramycin by itself. It has been demonstrated that fluoroquinolones are more efficient than cephalosporin, ß-lactams, and macrolides in preventing OM from non-typeable *H. influenzae* and also eliminating bacteria placed in the mushroom cap [23], [130]. Against respiratory tract bacteria such as *P. aeruginosa* or *S. pneumonia*, fluoroquinolones, particularly ciprofloxacin, and levofloxacin, have demonstrated efficacy [23], [75]. Combining phosphonic acid derivative fosfomycin, which has antibacterial effects on both Gram-positive and Gram-negative bacteria, with aminoglycosides (amikacin, isepamicin, netilmicin, tobramycin, or gentamicin) [75], [131]. When administered locally and at high quantities, the antibiotics ciprofloxacin, vancomycin, and imipenem were effective at breaking down bacterial biofilms that were firmly adhered. N-acetylcysteine (NAC) can lessen the adherence of respiratory pathogens to epithelial cells and prevent the development of *P. aeruginosa* and *S. aureus* biofilm [75].

6. Conclusion

Biofilm encompasses complex association of microorganisms, especially several types of bacteria engird within a self-made extracellular matrix that is made of a polymeric substance (EPS) with an altered physical and genetic appearance. Biofilm has the capability to trigger severe infections because it contains bacteria hiding and hibernating in a protective matrix and is reliable for 60-80% microbial infections. Moreover, it can stick to the ears, lungs, teeth, and other bodily systems which eventually cause serious illnesses. Bacterial biofilms cause a severe impendence on world health because they not only protect microorganisms from hostile environmental stress but also save bacterial colonies from being attacked by antibiotics and by the immune system of the host. Bacterial biofilms are linked to serious infections in the auditory, cardiovascular, digestive, reproductive, respiratory, urinary system, etc. Various diagnostic methods have also been used to understand the seriousness of these diseases such as; PCR, FISH, ELISA, SEM, mass spectrometry, bacteriological tests, CT scans, microbiological analyses, etc. Besides, there are several approaches that aid to control biofilm-related infection, for example, natural antimicrobial compounds, quorum sensing inhibitors, bacteriophages, DNase therapy, nanoparticles etc. This study focuses on understanding better how biofilms contribute to the progression of different diseases, as well as the different antibiofilm approaches.

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