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Review Article

Microbiology Section

A Narrative Review on Microbial Biofilm Formation in Septicaemia due to Gram-negative Bacteria: A Cause of Concern

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ABSTRACT

Septicaemia, or bloodstream infection, is a serious condition associated with high morbidity and mortality. Gram-negative bacteria, particularly Enterobacterales, are the primary causative agents of septicaemia. Globally, there is an increasing prevalence of antibiotic-resistant gram-negative bacteria reported in bloodstream infections. One of the major mechanisms of antibiotic resistance in gram-negative bacteria is their ability to form biofilms. Biofilms facilitate the transfer of Antimicrobial Resistance (AMR) genes among the bacteria present within the biofilm. Biofilm formation poses a challenge for treatment management; additionally, biofilms protect the bacteria from antibiotics and the host immune response, thus helping the organisms to establish resistance to antibiotic agents. To date, no conclusive strategies or appropriate agents are available to combat bacteria in microbial biofilms formed inside the human host. The best way to fight biofilm-forming bacteria is to prevent infection and eradicate it before biofilms are formed by following proper preventive measures and using appropriate antibiotics. This review article aims to help readers understand the complex relationship between biofilm-forming ability and AMR among gram-negative bacteria causing septicaemia. Furthermore, the review explores the impact of biofilm formation on the pathogenesis of septicaemia and discusses strategies and agents to prevent and combat biofilm formation.

Keywords: Antimicrobial resistance, Biofilm formation, Bloodstream infection, Enterobacterales

INTRODUCTION

Septicaemia, or bloodstream infection, poses a serious risk to life and has significant morbidity and mortality rates. Management of septicaemia is becoming increasingly difficult due to the continuously evolving Multidrug-Resistant (MDR) strains of bacteria that cause septicaemia, including Enterobacterales [1-3]. Among the Enterobacterales, *Escherichia coli* and *Klebsiella pneumoniae* are frequently detected as the causative organisms for septicaemia, and these organisms can form biofilms [3]. The emergence and global spread of Enterobacterales resistant to antibiotics is a serious problem. Enterobacterales that produce carbapenemase or Extended-Spectrum Beta-Lactamases (ESBL) can result in both hospital-acquired and community-acquired septicaemia [3]. Treatment for sepsis caused by antibiotic-resistant Enterobacterales is challenging [4]. Biofilm formation causes bacteria to become more resistant to antibiotics and bodily defense mechanisms [5]. Bacteria residing in the biofilm formed at the original infection site or on contaminated medical equipment can spread into the bloodstream, leading to septicaemia or sepsis [6,7]. Preventing infection with these organisms is crucial and can be achieved through the implementation of appropriate infection control practices.

Bloodstream Infection: Definition and Epidemiology

Septicaemia occurs when bacteria spread and actively grow in the circulation, producing toxins that overwhelm the host immune system and injure the host [1,2]. Enterobacterales are among the common organisms that cause septicaemia [3]. According to data published in 2020, there were 48.9 million incident cases and 11 million sepsis-related deaths worldwide, representing 20% of all global deaths in 2017. In the same year, there were 11 million incident cases of sepsis in India, resulting in nearly three million deaths. Of the global total, almost half (20.3 million) of the incident cases of sepsis occurred in children under five years of age [8]. Countries with low, low-middle, or middle socio-demographic indices experience higher rates of sepsis and greater mortality compared to high-income countries. In 2017, there were 17 million incident cases of sepsis and 3.5 million deaths from sepsis in Africa [8]. In North America and Europe, the rate of bloodstream infections varies from 113 to 204 per 100,000 people [9].

Addressing the prevention, diagnosis and treatment of septicaemia in low and middle-income countries, such as India, is essential to improve outcomes. Sepsis remains a major contributor to neonatal mortality. According to a World Health Organisation (WHO) fact sheet, approximately 2.3 million neonates died globally during the newborn period in 2022. Sub-Saharan Africa had the highest neonatal mortality rate in the world at 27 deaths per 1,000 live births, followed by Central and Southern Asia, with a neonatal mortality rate of 21 deaths per 1,000 live births (WHO 2024). From 1997 to 2016, out of the three million annual neonatal sepsis cases (2,202 per 100,000 live births), India had the highest incidence of clinical sepsis (17,000 per 100,000 live births) [10]. Fortunately, in India, the rate of neonatal sepsis has significantly declined from 111 cases per 1,000 live births in 1998 to 2001 to just 19 per 1,000 live births in 2016-2019 [11,12].

Causative Agents of Septicaemia

Bacteria, regardless of gram stain property, can cause septicaemia, with the causative agents varying by location, time and patient population [13]. In a study on hospital-acquired septicaemia in North India, gram-negative bacteria were the causative agents in one-third of the cases. The researchers suggested that the reasons for the lower incidence of gram-negative septicaemia may be due to a patient population with a male predominance and many patients having invasive devices as the causative factor for septicaemia [13]. In their study, Ramteke M et al., identified *Klebsiella pneumoniae* as the most common gram-negative bacteria causing septicaemia, isolated in 12.5% of the cases [14]. Bajaj A et al., reported that gram-negative bacteria were isolated from 65.8% of septicaemia cases [15]. *Klebsiella pneumoniae* was the most prevalent among Enterobacterales, while *Pseudomonas* spp. ranked highest among non fermenters. In a study of 357 blood samples from suspected cases of neonatal septicaemia, 154 samples tested positive for

bacterial growth, of which 62.3% were gram-negative. The most commonly isolated gram-negative bacteria were *Klebsiella* (64.5%) and non fermenting gram-negative bacteria (9.1%) [16]. Jyothi P et al., found that gram-negative bacilli caused 55.7% of neonatal septicaemia cases and *Klebsiella* spp. accounted for 30.5% of the gram-negative cases [17].

Understanding the significance of gram-negative septicaemia is crucial due to the prevalence of MDR bacteria [18], which are non susceptible to at least one agent in three or more antimicrobial categories and Extensively Drug-Resistant (XDR) bacteria, which are non susceptible to at least one agent in all but two or fewer antimicrobial categories, such as *Klebsiella pneumoniae*, *E. coli*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [18]. This resistance is often due to the acquisition of ESBLs, Oxacillinases (OXA), Verona Integron-encoded Metallo-beta-lactamase (VIM), New Delhi Metallo-beta-lactamase (NDM), *Klebsiella pneumoniae* Carbapenemase (KPC) and Imipenemase (IMP) enzyme-forming genes. The increase in MDR Enterobacterales causing septicaemia from 6.2% in 1997-2000 to 15.8% in 2013-2016 highlights the urgent need for effective management strategies [19]. Data from 2013-2019 reveals that *Klebsiella pneumoniae* was the top MDR pathogen responsible for bloodstream infections, followed by *E. coli* [19]. ESBL-producing Enterobacterales and carbapenem-resistant Enterobacterales can cause bloodstream infections and are linked to high mortality, especially in low-income countries [20]. In a study involving a total of 384 patients, 164 patients were found to be infected with Enterobacterales, out of which 44/164 (26.8%) patients had bloodstream infections. Of the 44 bloodstream infections with Enterobacterales, 11 cases were infected with Carbapenemase-Producing Carbapenem-Resistant Enterobacterales (CP-CRE). The most common CP-CRE isolates were *E. coli* and *K. pneumoniae* and all of them were resistant to multiple drugs [21]. In a study conducted in Morocco [22], the researchers discovered that 30% of sepsis cases in neonates were linked to Enterobacterales. Blood cultures identified 43 isolates of *K. pneumoniae*, 30 of *Enterobacter hormaechei*, 3 of *E. coli* and one case each of *Klebsiella aerogenes* and *Proteus mirabilis*. Most of these isolates were MDR and produced both ESBL and carbapenemase, such as OXA-48, NDM-1 and NDM-7. Furthermore, many of these Enterobacterales also had resistance genes for sulfonamide, trimethoprim and plasmid-modulated quinolone [22].

Age, Gender and Hospital Ward Distribution of Septicaemia

Sepsis is common in neonates and the elderly, with a decrease in childhood and an increase in adulthood, particularly in the 50 to 60 years age group. It is more prevalent in males in both neonates and the elderly [10,12,13]. Several factors are linked to a higher incidence of sepsis, including old age, male sex, being African American and various co-morbidities such as diabetes, chronic heart failure, chronic lung disease, immunocompromised status, chronic liver disease, malignancy and chronic kidney disease [23]. Hasnain A et al., identified diabetes, hypertension and smoking as significant risk factors for septicaemia [24]. Kabi A et al., pointed to immunosuppression, the use of invasive devices, being over 60 years old and severe injuries as major risk factors [13]. Mayr FB et al., noted that risk factors for septicaemia include older age, male sex, being African American, chronic health issues, poor economic conditions, residing in nursing homes, malnutrition, immunocompromised status, use of prostheses and hereditary predisposition [25].

In a study on neonatal septicaemia, Debnath J and Das PK, found that maternal factors such as fever, premature rupture of membranes, meconium-stained amniotic fluid, chorioamnionitis and maternal Urinary Tract Infections (UTI) are associated with neonatal septicaemia [26]. Additionally, male newborns, low birth weight and preterm birth also contribute to the risk. In one meta-analysis of 15 studies on sepsis in neonates in India, the researchers highlighted male sex, being born outside a healthcare facility, the need for artificial ventilation, preterm birth of less than 37 weeks and premature rupture of membranes as factors contributing to the development of septicaemia [12].

Infections in the respiratory tract, intra-abdominal area, urinary tract and bloodstream are the most common primary sources of infection that can lead to septicaemia. In their study, Chatterjee S et al., identified infections in the respiratory tract as the most common primary source of infection, accounting for 53.3% [27]. This was followed by intra-abdominal infections at 14.9%, bloodstream infections at 14.3%, UTI at 12.9% and infections from other sites, including skin, gynaecologic, central nervous system, unknown sources and bone/joint infections. Similarly, Esper AM et al., found that the primary sources of infection for sepsis were from the respiratory tract (33%), genitourinary tract (32%), gastrointestinal tract (23%), bone and joint (7%) and skin and soft-tissue infections (5%), with other sources making up 3% [28].

According to Xie J et al., 33.9% of sepsis cases were hospital-acquired, with lung infection being the most common site (68.2%), followed by abdominal infection (26.6%) and bloodstream infection (7.8%) [29]. In their research on septicaemia in older patients, Martin-Loeches I et al., found that pneumonia was the most common predisposing condition for sepsis, representing 39.8% of the total cases. This was followed by peritonitis at 35.6%, UTIs at 11.4% and skin and soft-tissue infections at 4.4% [30]. Kabi A et al., found that genitourinary infection was the most common primary source of infection for septicaemia in about 36.9% of the cases [13]. They noted that 74.9% of patients were from the medicine ward, with 17.1% from emergency wards and 8.0% from surgical wards. Martin-Loeches I et al., found that at the time of sepsis diagnosis, 46.4% of the patients were in the wards, 40% were in the emergency department and 13.6% were in the intensive care units [30]. Page DB et al., found that the rate of hospitalisation was higher in medical wards than in surgical wards among patients with severe sepsis acquired in the community, healthcare settings and hospitals [31].

What is biofilm? Implication of Biofilm and Biofilm Production

Antony Van Leeuwenhoek was the first to observe a mass of microorganisms, now recognised as biofilms, using his microscope. The term “biofilm” was introduced and defined by Costerton JW et al., biofilms consist of complex communities of microorganisms that adhere to surfaces and are surrounded by a self-produced matrix of Extracellular Polymeric Substances (EPS) [32-34]. In a biofilm, bacteria are sessile and drive the majority of processes within that environment. Due to their adaptation to microenvironments, they exhibit unique growth, gene expression and functional characteristics, leading to a viscoelastic structure with rubber-like properties [35,36]. The majority of human microbial infections are related to biofilm formation and Enterobacterales are commonly found within these biofilms [37].

The formation of biofilms involves multiple stages, including attachment, irreversible adhesion, microcolony formation, growth, maturation and dispersion. It is regulated by factors such as quorum sensing, two-component regulatory systems and intracellular signaling molecules. These mechanisms coordinate biofilm formation by responding to environmental cues and genetic components [38]. Human tissues aren’t the only surfaces on which bacteria form biofilms. Bacteria also adhere to indwelling medical devices, particularly in the blood circulatory system, potentially leading to septicaemia. Biofilms consist of multiple species of bacteria and Enterobacterales are commonly found in these biofilms [39,40].

Biofilms provide strong protection for microbial organisms in harsh environments, shielding them from harm and promoting the development of persistent infection sites that are difficult to eliminate. Bacteria in a biofilm can be up to a thousand times more resistant to antimicrobial agents compared to free-floating bacteria in cultures. Achieving a sufficiently high concentration of antibiotics to eliminate a mature biofilm within a living organism's body is not feasible [41-43]. The ability of bacteria to form biofilms depends on the organism and its environment. The amount of biofilm production by organisms also varies widely in the literature. Surgers L et al., conducted a study on biofilm formation by *E. coli* and *K. pneumoniae* strains producing ESBL enzymes [44]. They found that 57.1% of the organisms that produced ESBL were strong biofilm formers. About 61% of the *E. coli* strains showed standard biofilm production and strains that were ST131 clones produced biofilms less frequently compared to those strains possessing virulent factor genes of toxins and adhesins. About 90% of the *K. pneumoniae* strains produced standard biofilm and biofilm production was more frequently observed in ST29/147/323 strains than in other ST types. Ajaya B et al., demonstrated that among the Enterobacterales, 83/146 (56.85%) produced biofilms. *K. pneumoniae* and *Citrobacter* spp. had the highest rates of biofilm production, 17/24 (70.83%) and 29/57 (50.88%), respectively. Among the non fermenters, 25/42 (59.2%) isolates produced biofilms, with *Acinetobacter calcoaceticus-baumannii* complex showing a rate of 16/26 (61.5%) and *Pseudomonas* spp. showing a rate of 9/16 (56.25%) [45].

Biofilm detection: Methods, Sensitivity and Specificity of Different Methods

Many methods are available to detect the production of biofilm, such as the tissue culture plate method, tube method, Congo red agar method, crystal violet assay, bioluminescent assay, scanning electron microscopy, fluorescent in-situ hybridisation, confocal scanning laser microscopy, infrared spectroscopy and piezoelectric sensors. In microbiology laboratories, the tissue culture plate method is considered the gold standard for detecting biofilm. [Table/Fig-1] shows a comparison of various methods for detecting biofilm formation observed in three studies [45-47]. In the study by Mathur T et al., the tube method was less accurate compared to the modified tissue culture plate method, as the tube method could easily detect strong biofilm formers but had difficulty differentiating between moderate and weak biofilm formers [47].

Research study done by	Method of detection	Sensitivity	Specificity	Accuracy to differentiate biofilm formation and none formation
Ajaya B et al., [45]	Tissue culture plate method (Gold standard)			
	Tube method	90.8%	70.1%	
	Congo red agar method	68.2%	42%	
	Modified congo red agar method	65.1%	40%	
Shenoy V et al., [46]	Tissue culture plate method (Gold standard)			
	Tube method	47%	100%	
	Congo red agar method	56.9%	50%	
Mathur T et al., [47]	Standard tissue culture plate method (Gold standard)			
	Modified tissue culture plate method	96.2%	94.5%	97.3%
	Tube method	77.9%	96.8%	86.8%
	Congo red agar method	7.6%	96.2%	51.3%

[Table/Fig-1]: Studies of comparison of methods of detection of biofilm [45-47].

Biofilm formation: Relation to Antibiotic Resistance and Virulence

Biofilm is a natural protective mechanism of the organism. Recalcitrance is the phenomenon by which bacteria residing in a biofilm can withstand and survive very high concentrations of antibiotics [48]. The formation of biofilms by MDR bacteria can lead to increased antibiotic resistance and treatment failures in clinical settings. Biofilm renders an organism more resistant to antimicrobial agents, serves as a reservoir for genes responsible for resistance to these agents and facilitates the transfer of these genes among bacteria residing within the biofilm [49].

Enterobacterales, such as *E. coli*, *K. pneumoniae* and *P. mirabilis*, cause nosocomial infections through biofilms on implants, catheters and medical devices, serving as sources of infection [50,51]. Studies have shown that *E. coli* and *Klebsiella* strains, which possess the ability to form biofilms, are more likely to be antibiotic-resistant. However, for most *Enterobacter* species, the relationship between resistance to antibiotics and the ability to form biofilms remains unclear [52]. Qian W et al., found that antibiotic-resistant *E. coli* strains capable of producing biofilms have higher overall resistance and can cause treatment failure [4]. *E. coli* strains that formed strong and medium biofilms were mostly Extensive Drug Resistance (XDR), while the number of strains that formed weak biofilms was the same between MDR and XDR strains. Katongole P et al., also observed that strong biofilm-forming *E. coli* strains were usually XDR strains rather than MDR or non resistant strains and *E. coli* strains with the ability to form biofilms were more resistant to antibiotics than those strains that did not produce biofilms [53].

Tajbakhsh E et al., reported that among 130 *E. coli* strains isolated from cases of UTI, 61.53% were able to form biofilms [54]. The isolates showed the highest antibiotic resistance to ampicillin, followed by tetracycline, nalidixic acid and cotrimoxazole, with the lowest resistance observed against nitrofurantoin. Strong biofilm-forming isolates also possessed virulence genes, *fimH*, *pap*, *sfa* and *afa*, in 93%, 87%, 87% and 67% of the isolates, respectively. Oyardi O et al., investigated AMR and biofilm formation among gram-negative organisms isolated from clinical specimens [5]. They found that a major portion of the isolates were Enterobacterales, including *K. pneumoniae*, *E. coli* and *Enterobacter* strains, which were capable of biofilm formation and multidrug resistance.

The relationship between the production of biofilm by an organism and the pattern of antibiotic resistance in that organism is complex and requires further research. A study from China suggested a possible link between the ability to produce biofilm and the pattern of antibiotic susceptibility. The study evaluated the correlation between biofilm production and susceptibility to eight antibiotic groups among *E. coli* isolates, finding that isolates resistant to six antibiotic groups formed stronger biofilms. However, there was no significant difference in biofilm formation capacity between susceptible and resistant isolates against β-lactams and lipopeptides [4].

Fabrega A et al., showed that, in the case of *Salmonella enterica*, biofilm formation is inversely correlated with susceptibility to quinolone antibiotics [55]. In a study conducted in India, it was found that a significant percentage of Enterobacterales causing septicaemia were biofilm-forming. Among *E.coli* isolates, 23.29% and among *K. pneumoniae* isolates, 39.73%, were capable of producing biofilm. These strains that were capable of forming biofilm were highly resistant to certain antibiotics, but there was no significant relationship between their ability to form biofilm and the pattern of antibiotic susceptibility [6].

In a study from Egypt, out of 45 strains isolated from septic neonates, eight were Enterobacterales, equally distributed between *E. coli* and *K. pneumoniae*. All the *K. pneumoniae* and half of the *E. coli* were biofilm formers and showed high resistance to multiple antibiotics [7]. Dumaru R et al., conducted a study in Nepal showing that Enterobacterales were a major part of the gram-negative isolates from clinical samples, including blood [56]. The majority of the isolates were biofilm-forming: *Klebsiella* spp. (77.55%), *Pseudomonas* spp. (73.68%), *E. coli* (60.33%), *Enterobacter* spp. (59.26%) and *Citrobacter* spp. (62.50%), having the highest rates of biofilm formation.

The isolated organisms showed significant ESBL and MBL production. *E. coli* exhibited 38.1% ESBL and 9.09% MBL, *K. pneumoniae* showed 30.61% ESBL and 26.53% MBL, *Acinetobacter* spp. showed 15.87% ESBL and 20.63% MBL, *Pseudomonas* spp. showed 15.79% ESBL and 26.31% MBL, *Enterobacter* spp. showed 7.41% ESBL and 11.11% MBL, *Citrobacter* spp. showed 25% ESBL, *Proteus* spp. showed 40% ESBL and 20% MBL and *Providencia stuartii* showed 100% ESBL production. ESBL producers were mostly biofilm-forming, but the association was not significant. However, there was a significant association between the production of MBL enzymes and the ability to produce biofilms by the organisms.

Al-Bayati M and Samarasinghe S, found that the *E. coli* IMP strain, which is resistant to carbapenem and the carbapenem-resistant strain of *K. pneumoniae* NDM produced more biofilm compared to non resistant strains [57]. During the mid-adhesion phase of biofilm formation, there was maximum upregulation of the genes responsible for biofilm production and the genes responsible for resistance to antibiotics. [Table/Fig-2] summarises the relationship between the ability to produce biofilm, the pattern of antimicrobial susceptibility, antimicrobial enzyme production and the presence of virulent genes among gram-negative bacteria [4,6,7,53,54,56].

Measures to Prevent Biofilm Formation, by Gram Negative Bacteria Causing Septicaemia

Strategies and therapeutic agents are needed to address treatment failure in bacterial infections associated with biofilm formation. When selecting treatment for bacterial infections associated with biofilm formation, we need to understand the mechanisms by which these organisms are recalcitrant. The early stage of biofilm formation is reversible, while established biofilms can be reduced or eliminated using various methods such as EPS antagonists, dissociation drivers, vaccination treatments and mechanical elimination. The production of EPS in biofilms is a dynamic process, making the biofilm resistant to different antibiotics. Efflux pumps, which are transport proteins present in the bacterial cell membrane, move antibiotics out of the cell, resulting in antibiotic resistance and also aiding in biofilm formation. Efflux pump inhibitors can render the organism susceptible to antibiotics.

Strategies against bacterial infections associated with biofilm formation include the topical administration of high-concentration antibiotics, combined antibiotic administration and the use of antibiotic adjuvants. A properly selected combination of antibiotics is more effective than a single antibiotic for treating these infections. The composition, sorption properties and charge of the extracellular matrix proteins of the biofilm influence resistance to antimicrobials and these factors should be considered when selecting antibiotics for treatment [58-60].

Several non antibiotic agents are known to inhibit and/or eradicate biofilms. These agents can act at different levels as biofilm inhibitors, biofilm dispersers and antimicrobials. They include antimicrobial peptides, bacteriophages, nanoparticles, QS system inhibitors, monoclonal antibodies, natural products and probiotics [52,61]. Various natural products are known to have antibiofilm activities, as shown in [Table/Fig-3] [61-64]. Enhancing the host immune response is crucial in combating biofilm-forming, antimicrobial-resistant bacteria.

Studies	Isolates	Biofilm production		Antimicrobial Resistance (AMR)			Enzyme production and virulence genes
				No MDR	MDR	XDR	
Qian W et al., [4]	*E. coli*, 81 isolates	Strong	46 (56.8%)	1 (2.2%)	9 (19.6%)	36 (78.3%)	
		Moderate	15 (18.5%)		5 (33.3%)	10 (66.7%)	
		Weak	8 (9.9%)		4 (50%)	4 (50%)	
		None	12 (14.8%)		7 (58.3%)	5 (41.7%)	
Juliana A et al., [6]	*K. pneumoniae*	29 (39.73%)			Most isolates		
	E. coli	17 (23.29%)					
	Acinetobacter spp.	25 (34.25%)					
Ebrahem AM et al., [7]	*K. pneumoniae*, 4 isolates	2 (50%), Strong				Highly resistant to all the tested antibiotics	
		2 (50%), Moderate					
	E. coli, 4 isolates	2 (50%), Moderate					
		2 (50%), None					
Katongole P et al., [53]	Uropathogenic *E. coli*, 200 isolates	Biofilm forming 125 (62.5%)			Significant likelihood for biofilm forming isolates		Biofilm formers had more adhesin genes like *fim*, *pap*, *sfa* and *afa*. Statistically not significant.
Tajbakhsh E et al., [54]	Uropathogenic *E. coli*, 130 isolates	Biofilm forming 80/130 (61.5%)	Strong 15 (18.75%)			Biofilm producers were less susceptible to 9 antibiotics group tested	Significantly more prevalence of *fimH*, *pap*, *sfa* and *afa* genes among high biofilm formers than less biofilm formers.
			Medium 20 (25%)				
			Weak 45 (56.25%)				
Dumaru R et al., [56]	GNB, 314 isolates	197 (62.7%), biofilm formers, *Klebsiella* spp. (77.5%, biofilm forming), *Pseudomonas* spp. (76.3%, biofilm forming), *E. coli* (60.3%, biofilm forming)					*Klebsiella* spp. 15/49 (30.61%) ESBL and 13/49 (26.53%) MBL. *Pseudomonas* spp. 6/38 (15.79%) ESBL and 10/39 (26.31%) MBL, *E. coli* 46/121 (38.1%) ESBL and 11/121 (9.09%) MBL.

[Table/Fig-2]: Relationship between biofilm forming ability, AMR and virulence [4,6,7,53,54,56].

Natural products having antibiofilm activity	Mechanism of action	Target organisms
Epigallocatechingallate (EGCG), Reserpine, Quercetin, Linoleic acid, Berberine, Chitosan, Eugenol, Curcumin	Inhibits biofilm formation	*P. aeruginosa*, K. pneumonia, *E. coli* and *Acinetobacter baumannii*

Antimicrobial peptides like LL-37, Lytic peptide (PTP-7), PMAP-23	Neutralisation and disaggregation of lipopolysaccharides	*P. aeruginosa* and *E. coli*
Ebselen and Ebselen oxide	Inhibits DGC activity and limits c-di-GMP binding	Gram-negative bacteria
Maipomycin A	Derivative of K. phytohabitans xy-210, inhibits biofilm formation	Gram-negative bacteria and also enhances the effect of colistin against *A. baumannii*
Chinese ginseng, garlic and Azithromycin	Inhibits quorum sensing	*P. aeruginosa*

[Table/Fig-3]: Natural products having antibiofilm activity [61-64].

CONCLUSION(S)

Gram-negative bacteria, particularly Enterobacterales, that have the ability to form biofilms and are resistant to antibiotics are becoming a major cause of septicaemia and are associated with treatment failure and poor prognosis. Understanding the mechanisms of biofilm formation and developing agents and approaches to prevent biofilm formation, as well as disrupting or disintegrating formed biofilms, is crucial for formulating effective treatment methods. It is high time to develop novel antimicrobial agents and innovative strategies that are effective against these organisms, which are resistant to antimicrobials and form biofilms.

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