ORIGINAL ARTICLE

Antibiofilm Activity of Naproxen against Bacterial Isolates from Sinusitis and Tonsillitis Patients

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ABSTRACT

Background: *Biofilm formation is a significant virulence factor in bacterial infections, contributing to antibiotic resistance and chronic infections.* ***Objective:*** *Its aims to investigated the microbial profile of sinusitis and tonsillitis infections and evaluated the antibacterial and antibiofilm effects of naproxen against clinical isolates.* ***Methodology:*** *A total of 126 swab samples (42 sinusitis, 84 tonsillitis) were collected and cultured on selective media, yielding 366 bacterial isolates identified by Vitek 2 system. Predominant species included Staphylococcus aureus (35.5%), Pseudomonas aeruginosa (23.5%), and Streptococcus pyogenes (11.7%), with significant species-specific infection associations ($p<0.05$).* ***Results:*** *Biofilm formation assessed via Congo Red Agar revealed 59.3% of isolates were biofilm producers (97 strong, 120 moderate). Naproxen demonstrated concentration-dependent antibacterial activity, showing greatest efficacy against Gram-positive cocci (inhibition zones 16-30 mm) but limited activity against P. aeruginosa and P. putida at concentrations ≤1000 µg/mL. Notably, sub-inhibitory naproxen concentrations significantly reduced biofilm formation in treated isolates compared to controls ($p<0.01$).* ***Conclusion:*** *Naproxen possesses both antimicrobial and biofilm-disrupting properties, supporting its potential therapeutic utility in biofilm-associated respiratory infections.*

INTRODUCTION

Chronic rhinosinusitis and recurrent tonsillitis are widespread and challenging conditions of the upper respiratory tract, often marked by ongoing inflammation, damage to the mucosal lining, and repetitive episodes of infection and symptom flare-ups. Despite the routine administration of antibiotics, corticosteroids, and even surgical procedures, therapeutic success is frequently limited, and relapses are common, presenting ongoing difficulties for both clinicians and patients. Mounting evidence indicates that this persistence is not solely due to the virulence of the pathogens or shortcomings in host immunity, but is also strongly associated with the presence of biofilm-producing bacteria, which exhibit substantial resistance to conventional treatment modalities[1].

Biofilms are organized aggregates of microorganisms encased within a self-generated extracellular matrix composed of complex biopolymers, including polysaccharides, proteins, lipids and nucleic acids. This matrix serves as both a physical and chemical shield, hindering immune system attacks and reducing the effectiveness of antimicrobial agents[2]. Furthermore, bacteria within these structures undergo physiological alterations-such as decreased metabolic activity, changes in gene expression and a shift to a dormant state-that collectively enhance their resilience against antibiotics that would otherwise kill their free-floating (planktonic) counterparts[3]. These biofilm-specific adaptations make infections much harder to eliminate, often requiring extended or repeated interventions and increasing the risk of chronic tissue inflammation and irreversible damage.

Several key bacterial species known to be clinically significant have been implicated in the formation of biofilms in the upper respiratory tract. Among them, *Staphylococcus aureus , Streptococcus pyogenes, Haemophilus influenzae and Pseudomonas aeruginosa* are frequently recovered from patients suffering from chronic sinus and tonsil infections[4]. These pathogens not only establish stable biofilms on epithelial surfaces but also contribute to the persistence of clinical symptoms, including nasal congestion, mucopurulent discharge, throat pain and difficult swallowing. Their remarkable ability to evade immune responses and resist antibiotics underscores the urgent need for novel therapeutic strategies that directly disrupt biofilm structure and function.

The global increase in antimicrobial resistance (AMR) has further emphasized the importance of developing alternative or complementary treatments that

extend beyond the scope of traditional antibiotics. One emerging strategy involves the repurposing of established non-antibiotic drugs that possess previously unrecognized antimicrobial or anti-biofilm capabilities. In this context, non-steroidal anti-inflammatory drugs (NSAIDs) have gained attention-not only for their pain-relieving and anti-inflammatory effects but also for their potential to interfere with bacterial virulence mechanisms[2]. Research suggests that NSAIDs can impair various bacterial behaviors, including motility, the production of virulence factors, and quorum sensing-a bacterial communication process that plays a key role in coordinating biofilm development[5].

Among the NSAIDs, naproxen has demonstrated promising anti-biofilm effects in vitro. Laboratory studies have shown that naproxen can inhibit the formation of new biofilms and also disrupt mature biofilms in multiple bacterial species[6]. This activity is thought to be mediated by interference with bacterial gene expression, inhibition of the synthesis of extracellular matrix components, and disruption of quorum sensing pathways such as those involving acyl-homoserine lactones[7]. These findings point to a potential dual-function for naproxen-not only mitigating inflammation but also acting directly on bacterial communities that are otherwise difficult to treat.

Nevertheless, the efficacy of naproxen against bacterial isolates specifically obtained from patients with chronic sinusitis and recurrent tonsillitis has not been thoroughly examined. Much of the current research has relied on reference laboratory strains or pathogens unrelated to upper respiratory tract infections, limiting its relevance to real-world clinical contexts. Given the distinct physiological conditions in the upper respiratory tract-including variations in local immunity, pH, oxygen levels and microbial flora-it is essential to determine whether naproxen maintains similar anti-biofilm effects under these conditions.

Accordingly, this study aims to vigorously evaluate the biofilm-inhibiting and -disrupting properties of naproxen using in vitro models with bacterial strains isolated directly from patients affected by chronic sinus and tonsil infections. By analyzing naproxen's impact on biofilm mass, architecture, and bacterial viability, this research seeks to determine its potential as a supplementary treatment option. The results may provide a foundation for integrating anti-biofilm agents into current therapeutic protocols, with the ultimate goal of improving outcomes in biofilm-associated infections of the upper respiratory tract.

METHODOLOGY

Sample Collection and Bacterial Isolation

One hundred and twenty six swab samples were collected from patients (42 sinusitis, 84 tonsillitis). Samples were streaked on MacConkey, Mannitol Salt, and Chocolate agar plates and incubated at 37°C for 24 hours. Isolates were identified using the Vitek 2 system (bioMérieux, France).

Biofilm Formation Assay

Biofilm production was evaluated via Congo Red Agar (CRA) according to Freeman *et al.* [8] with modifications including Brain Heart Infusion Agar (37 g/L) supplemented with 0.8 g/L Congo Red and 50 g/L sucrose. Biofilm-positive strains exhibited black (strong) or pink (moderate) colonies, while non-biofilm formers remained red.

Naproxen Extraction

10 mg of naproxen was dissolved in methanol, filtered, and dried to obtain a powder.

Concentration Preparation

Solutions of 100, 1000, 10,000, and 100,000 µg/mL were prepared using dimethyl sulfoxide (DMSO).

Well Diffusion Assay

Bacterial suspensions were spread on Mueller-Hinton agar, and wells were filled with naproxen solutions. Inhibition zones were measured after incubation for 24 hours at 37 ^{0}C.

Antibiofilm activity

This experiment was determined by picking up colonies from the margin of inhibition zone in which bacteria were exposed to naproxen but not killed and the control were selected from the colonies that not exposed to naproxen. The colonies were then streaked on Congo Red Agar

Statistical analysis

The data were analyzed using SPSS version 28. The statistical significance of the difference in data was assessed by Chi-square, p-values $\leq$ 0.05 were considered statistically significant

RESULTS

A total of 366 bacterial colonies were obtained from culturing media used in this study. Results of Vitek 2 analyses showed that the isolated bacteria belong to the following species (130 isolates belong to *Staphylococcus aureus* (with accuracy of 99%), 32 isolates were *Staphylococcus epidermidis* (98%), 36 isolates were to *Streptococcus thoraltensis* (98%), 39 isolates to *Pseudomonas putida* (98%), 86 were *Pseudomonas aeruginosa* (99% and 43 were *Streptococcus pyogenes* 99%). Statistical analyses showed a significant association between bacterial species and infection type (p<0.05), and that *Ps. aeruginosa* is more linked to sinusitis, while *S. aureus* is more linked to tonsillitis (Table 1).

The results of biofilm activities of identified bacteria showed different levels of biofilm formation according to the appearance of colonies on Congo red agar (97 isolates were strong former belongings to the conversion of Congo red pigment into black, 120 isolates were moderate biofilm former that appeared

mucoid and pink, 26 isolate were weak former that appear pink but not mucoid, while the 126 isolates showed no biofilm activities that appeared red and flat colonies (Fig. 1).

Naproxen with different concentrations exhibited different inhibitions against studied bacteria. Statistical analyses showed that *P. aeruginosa* and *P. putida* showed high resistance to naproxen (at lower concentrations), while *S. pyogenes* and *Staph. epidermidis* were more sensitive. The results of antibacterial activities of naproxen were listed in (Table 2 and Fig. 2).

Table 1: Distribution of microorganisms isolated from patients with sinusitis and tonsilitis ($p<0.05$)

Bacterial Species	Tonsilitis		Sinusitis		Total	
	number	%	number	%	number	%
Staphylococcus aureus	100	27.32	30	8.20	130	35.52
Pseudomonas aeruginosa	24	6.567	62	16.94	86	23.50
Streptococcus pyogenes	28	7.65	15	4.10	43	11.75
Pseudomonas putida	22	6.01	17	4.65	39	10.66
Streptococcus thoraltensis	29	7.92	7	1.91	36	9.84
Staphylococcus epidermidis	8	2.19	24	6.56	32	8.74
Total	211	57.65	155	42.35	366	100

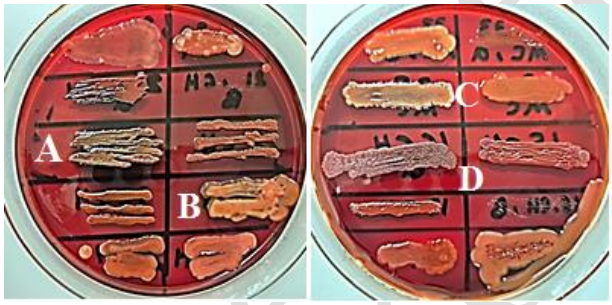

Fig. 1 : Different levels of biofilm formation exhibited by different bacterial isolates. (A=strong former, B= moderate biofilm former, C= weak former and D= no biofilm formation)

Table 2: Effect of different concentration of naproxen on isolated bacteria ($p<0.01$)

Bacterial Species	Naproxen Con. (µg/ml)			
	100	1000	10000	10000
	Diameter of inhibition zone (mm)			
Staphylococcus aureus	16	17	25	28
Pseudomonas aeruginosa	0	0	14	16
Streptococcus pyogenes	22	23	23	24
Pseudomonas putida	0	12	20	22
Streptococcus thoraltensis	12	16	20	30
Staphylococcus epidermidis	20	22	22	30

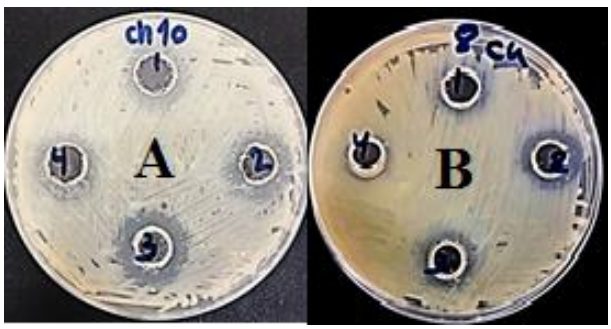

Fig. 2 : Antibacterial effect of naproxen against (A=*Staphylococcus epidermidis* , *B= Streptococcus thoraltensis* , *C= Pseudomonas aeruginosa and D= Pseudomonas putida*)

The technique used to assess the ability of the isolated bacteria to produce biofilm is a qualitative method that used Congo red agar (CRA) medium, in this method, exposed bacteria to naproxen were selected by picking up colonies from the margins of inhibition zones from muller Hinton medium and streaked on (CRA) and the control was taken from the area not exposed to naproxen, the result showed that exposed bacteria to naproxen lost the character of biofilm synthesis or became more vulnerable (Fig. 3).

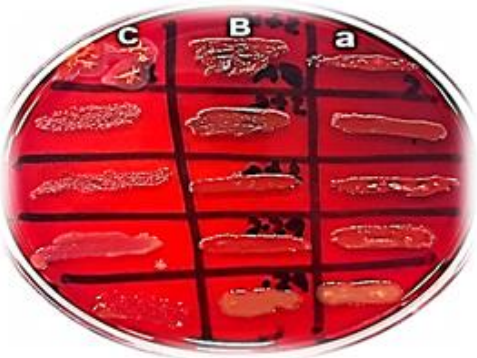

Fig. 3: Antibiofilm activity of naproxen against biofilm forming bacteria (a & b control and C exposed bacteria)

DISCUSSION

The present study investigated the antibacterial and antibiofilm effects of naproxen against clinically relevant bacterial isolates from sinusitis and tonsillitis patients. Our findings reveal significant associations between bacterial species and infection type, differential biofilm-forming capabilities, and concentration-dependent antimicrobial activity of naproxen.

The predominance of *S. aureus* (35.5%) and *P. aeruginosa* (23.5%) aligns with previous reports linking *S. aureus* to tonsillitis and *P. aeruginosa* to chronic sinusitis[9]. The statistically significant Pseudomonas association (p<0.05, Chi-square test) between bacterial species and infection type suggests niche-specific adaptations, possibly due to differences in mucosal adherence and immune evasion mechanisms[10]. The high prevalence of *S. aureus* in tonsillitis aligns with its role as a primary pathogen in recurrent throat infections, often due to its ability to evade immune responses via protein A and biofilm formation[11]. Conversely, *P. aeruginosa* dominated in sinusitis, likely due to its adaptation to mucosal hypoxia and intrinsic resistance to host defenses[12]. The statistical significance of these associations underscores the need for infection-specific therapies.

Biofilm production, a key virulence factor, was observed in 59.3% of isolates, with *P. aeruginosa* and *S. aureus* being strong formers. This corroborates studies implicating biofilms in antibiotic resistance and chronic infections[13]. The Congo Red Agar (CRA) method effectively differentiated biofilm phenotypes, though quantitative assays (e.g., crystal violet) could further validate these findings[14].

Naproxen exhibited broad-spectrum inhibitory effects, particularly against Gram-positive cocci (*S. pyogenes* and *S. epidermidis*), consistent with studies showing NSAIDs disrupt bacterial membrane integrity[15]. In contrast, *P. aeruginosa* and *P. putida* displayed high resistance, likely due to efflux pumps and biofilm-mediated protection[16]. Gram-positive susceptibility: *Streptococcus pyogenes and S. epidermidis* were highly sensitive (inhibition zones: 20–24 mm at 100 µg/mL), likely due to naproxen's disruption of bacterial membrane potential[17]. Gram-negative resistance: *P. aeruginosa and P. putida* showed no inhibition at ≤1,000 µg/mL (table 2), attributable to efflux pumps (e.g., MexAB-OprM) and LPS barrier function[16].

Our data demonstrate that naproxen at sub-inhibitory doses attenuates biofilm formation, potentially by interfering with quorum-sensing pathways, e.g. las/rhl systems in *P. aeruginosa*, consistent with prior reports[18]. This supports repurposing NSAIDs as adjuvants to antibiotics in biofilm-associated infections. Naproxen-exposed isolates lost black/pink pigmentation on CRA (Fig. 3), indicating Downregulation of polysaccharide synthesis, e.g., pel and psl genes in *P. aeruginosa*[18].

CONCLUSION

This study identified *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the most common bacteria associated with chronic tonsillitis and sinusitis, respectively, with a significant link between infection type and bacterial species. Many isolates showed strong biofilm-forming abilities, which likely contribute to the persistence and treatment resistance of these infections. Naproxen demonstrated antibacterial activity, particularly against *Streptococcus pyogenes* and *Staphylococcus epidermidis*, while *P. aeruginosa* and *P. putida* were more resistant. Moreover, naproxen reduced or inhibited biofilm formation in exposed bacteria, indicating its potential as an adjunct therapy in managing biofilm-related infections.

Ethical Approval

Ethical approval to conduct to the study was obtained from the University of Basra Research Ethics Committee in accordance with Protocol No. EU 85 dated June 2, 2022. Verbal consent was obtained from all patients participating in the study.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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