



Efficacy of sodium hypochlorite on removal of biofilm produced by *P. aeruginosa* and *S. aureus*

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Abstract

Biofilms are complex microbial communities encased in a self-produced extracellular matrix, which contribute significantly to bacterial persistence and resistance to antimicrobial agents. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are among the most common biofilm-forming pathogens, often implicated in chronic infections and medical device-related complications. This study investigates the efficacy of sodium hypochlorite in disrupting and removing biofilms formed by these bacteria. Using quantitative analysis and microscopy techniques, we assessed the impact of different concentrations and exposure times of sodium hypochlorite on biofilm degradation. The results indicate that sodium hypochlorite effectively reduces biofilm biomass and disrupts bacterial viability, with higher concentrations showing a more pronounced effect. These findings underscore the potential of sodium hypochlorite as an effective biofilm control agent in healthcare and industrial settings.

Keywords: Sodium hypochlorite, biofilm, efficacy, *P. aeruginosa*, *S. aureus*

Introduction

Biofilm is a well natural designed structure of multi microbial cells embedded within a complex matrix of polysaccharides, proteins, nucleic acids and glycoproteins (Donlan and Costerton, 2002) [9]. Biofilm formation allows non-spore forming soil bacteria to colonize surrounding habitat and to survive common environmental stresses as nutrition limitation (Luciana and Giordano, 2010) [15]. Both Gram-positive and Gram-negative bacteria have the capability to form biofilms. According to a publication by the National Institute of Health, more than 80% of all infection involves biofilms (NIH, 2002). Bacteria commonly involved include *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus viridians*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* (Dolan, 2001) [8].

Biofilms are associated with many medical conditions including indwelling medical devices, dental plaque, upper respiratory tract infections, peritonitis, and urogenital infections (Reid, 1999) [16]. There are various methods to detect biofilm production. These includes Tissue Culture Plate (TCP), Tube method (TM), Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensors and fluorescent microscopic examination. (Hassan, 2011) [10].

Disinfectants are chemical agents used to inactivate all recognized microorganisms, the mode of action of disinfectants depends on biocide used, potential target sites in Gram positive or Gram-negative bacteria are the cell wall or outer membrane, cytoplasmic membrane, functional and structural protein, DNA, RNA and other cytosolic components. Although biocide treatment eliminates most surface contamination some microorganisms may survive and give rise to public health problem (Bridier *et al.*, 2011) [7].

Antiseptics and disinfectants are used extensively in hospitals and other healthcare settings for a variety of topical and hard-surface applications. Sodium hypochlorite is one of the chemical compounds used for bleaching or

disinfecting different things including surfaces. The efficiency of disinfection operation depends on the microorganism, the disinfectant, the concentration of disinfectant, the contact time, the pH, the temperature, etc. The relationship between the concentration of the disinfectant and the contact time should be considered to achieve a determined reduction of the microorganism (Amzil *et al.*, 2019) [2].

Pseudomonas aeruginosa and *Staphylococcus aureus* both can form the biofilm which make it difficult for the control. Cleaning and disinfection are the keyway to prevent the contamination of the contact surfaces and consequently food, drinking products. Sodium hypochlorite, chlorohexidine, ethel alcohol, hydrogen peroxide, peracetic acid are some of the disinfectants used for inhibition of biofilm.

The objective of this study is the isolation and identification of biofilm producing *Staphylococcus aureus* and *Pseudomonas aeruginosa* from non-clinical samples from the hospital environment. The efficiency of sodium hypochlorite against the biofilm by using different parameters like the contact time, concentration of sodium hypochlorite.

Material and Methods

■ **Collection of Samples:** The sample collection was done from various hospital environment. The basin sample collection was done using the sterile cotton swab. The swab, medium and the test tubes were sterilized by autoclaving method. The use of Nutrient broth medium was done for the transport and storage of sample in a test tube. The sample from various hospitals like City Hospital, Ozone Hospital, Sahara Hospital, Lady Hardinge Hospital were collected.

■ **Isolation and Identification of *Staphylococcus aureus* and *Pseudomonas aeruginosa*:** From the collected basin sample the isolation of *Staphylococcus aureus* was done on Mannitol Salt Agar and isolation of

Pseudomonas aeruginosa on Cetrimide Agar. The samples were inoculated on their respective agar and incubated for 24 hrs at 37° C and after incubation bacterial growth were observed. The bacterial isolates were then sub-cultured on the Mannitol Salt Agar and Cetrimide Agar respectively, microscopic analysis and Biochemical tests were performed.

- **Screening of Biofilm Producing *Staphylococcus aureus* and *Pseudomonas aeruginosa*:** For the screening process the Congo Red Agar method (CRA) and Tube method (TM) was used.
- **Congo Red Agar Method:** In CRA method the medium was prepared using the BHI agar, the Congo red stain (concentrated aqueous solution) and sucrose. All media were separately autoclaved. In that autoclaved BHI agar, the Congo Red was added followed by the sucrose. The medium was poured into the autoclaved petri plates. After the solidification of the medium the isolated sample was inoculated onto the prepared medium and incubated for 36-48 hrs at 37° C. After incubation, the growth was observed with black coloured zones around the colony. The colour shows how strong the biofilm producing bacteria was in the samples. The darker or blackish colour shows the strong biofilm producing bacteria, the lighter or greyish colour of the agar shows weaker biofilm producing bacteria. The non-biofilm producing bacteria does not change the colour of agar.
- **Tube Method:** This was qualitative method for the detection of the biofilm. The loopful of culture was inoculated into the 1ml of nutrient broth into the test tube and incubated for 24 hrs at 37°C. After 24 hrs this culture medium was transferred into the 5ml of BHI broth again incubated for 24 hrs at 37°C. After incubation the culture was discarded and stained using the 0.1% of crystal violet was added into the tube for 20 minutes. After 20 minutes the crystal violet was removed and washed using the Phosphate buffer solution. The biofilm present on the surface test tube gets stained by the crystal violet. After drying, onto the surface of test tube the biofilm stained as purple colour was observed.
- **Determination of Biofilm Against the Activity of Sodium Hypochlorite:** In this method the loopful of culture was inoculated into the test tube containing 1ml of nutrient broth and incubated for 24hrs at 37°C. After 24 hrs. this culture medium was transferred into the 5ml of BHI broth and again incubated for 24hrs at 37°C. After incubation the culture was discarded and the different concentrations of Sodium Hypochlorite (1%,2%,4%) were added into the test tube for 5 minutes. The sodium hypochlorite was removed out from test tubes and the addition of Phosphate buffer solution was done for washing of test tubes. After that 0.1% of crystal violet was added into the tube for 20 minutes. After 20 minutes the crystal violet was removed and again washed using the Phosphate buffer solution. The biofilm present on the surface test tube

gets stained by the crystal violet and after drying the test tubes at room temperature the purple color was observed on the inner surface of test tubes. After that 95% of Ethanol added into the test tubes for the removal the crystal violet stained biofilm. After 5 Minutes the Optical Density (OD) of the ethanol was taken at 540nm wavelength.

- **Antibiotic Susceptibility Testing of *Staphylococcus aureus* and *Pseudomonas aeruginosa*:** Antibiotic susceptibility test of biofilm producing bacteria was done on Mueller Hinton agar using the Kirby-Bauer method/ Disc diffusion method. For Antibiotic Susceptibility Test following antibiotic discs were used: Gentamicin (GEN¹⁰), Erythromycin (E¹⁵), Ciprofloxacin (CIP¹⁰), Ampicillin (AMP¹⁰), Amoxycillin (AMC³⁰), Chloramphenicol (C³⁰).

Results and Discussion

In the present study a total of 33 non-clinical samples collected from wash basins of various sites of different hospitals of Akola city (Table 1). The prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was studied (Fig 1). It was found that out of 33 samples *Pseudomonas aeruginosa* was isolated from 17 (51.55%) samples while *Staphylococcus aureus* was isolated from 16 (48.45%) samples.

The morphological, cultural and biochemical characteristics of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were determined using different morphological, cultural and biochemical methods (Table 2). For the screening of biofilm producing *Pseudomonas aeruginosa* and *Staphylococcus aureus* two methods were used i.e. Tube Method and Congo Red Agar Method. In tube method the biofilm producer *Pseudomonas aeruginosa* was found to be only 6 (35.2%) out of 17 and for *Staphylococcus aureus* biofilm producer was found to be 14 (87.5%) out of 16 (Table 3). By Congo Red Method for screening of biofilm producing isolates of *Pseudomonas aeruginosa* was found to be 6 (35.2%) out of 17 and for *Staphylococcus aureus* biofilm producer was found to be 14 (87.5%) out of 16 (Table 4).

The antibiotic susceptibility tests were performed on a total of 33 isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The results for *Pseudomonas aeruginosa* showed that 17 isolates were sensitive to both Ciprofloxacin and Gentamicin. Only 3 isolates were sensitive to Erythromycin, while the rest were resistant. Ampicillin was found to be resistant amongst all isolates, and Chloramphenicol was sensitive towards 8 isolates. For *Staphylococcus aureus*, it was observed that 10 isolates were sensitive to Ciprofloxacin, and 13 isolates were sensitive to Gentamicin. Erythromycin and Chloramphenicol were sensitive amongst 4 and 10 isolates, respectively. All isolates of *Staphylococcus aureus* were resistant to Ampicillin (Table 5).

The efficacy of Sodium hypochlorite on the biofilm removal was measured by using the colorimetric analysis. The Optical Density (OD) at 540 nm was recorded for the different concentrations of Sodium Hypochlorite 1%, 2% & 4% at different contact times of 1 min, 3 min and 5 min. Among the isolates of *Pseudomonas aeruginosa* 6 isolates were showing the production of biofilm (Fig 2-4). The

Isolate P1 of *Pseudomonas aeruginosa* demonstrated the maximum removal of biofilm at the 4% concentration of sodium hypochlorite with a contact time of 5 minutes, where the optical density (OD) was found to be 0.33. The minimum removal was observed at the 1% concentration with a contact time of 1 minute, where the OD was recorded as 0.07. The amount of biofilm removal by the 2% concentration at 5 min was also effective as it also demonstrated the OD of 0.30. For Isolate P2 the highest biofilm removal at the 4% sodium hypochlorite concentration with a contact time of 5 minutes, yielding an OD of 0.44. In contrast, the 1% concentration at 1 minute showed the least removal, with an OD of 0.04. The maximum biofilm removal at 4% sodium hypochlorite concentration and 5 minutes contact time was also exhibited on the isolate P3, with an OD of 1.56, indicating a strong biofilm reduction. The least removal was seen at 1% concentration and 1 minute, with an OD of 0.09. By the concentration of 4% at contact time of 1 minute, the removal of biofilm is more than that of the contact time of 5 minutes of concentration of 1% and 2% exhibiting the OD of 0.36 compared to 0.25 and 0.33 respectively.

Isolate P4 exhibited the highest removal of biofilm at 4% concentration after 5 minutes, with an OD of 0.37. At 1% concentration for 1 minute, the biofilm removal was the lowest, with an OD of 0.05. The 4% concentration of contact time 1.3 minute showed the comparable removal of biofilm against 5 minutes contact time of 1% and 2% respectively. Isolate P5 demonstrated the greatest biofilm removal at the 4% sodium hypochlorite concentration and 5 minutes contact time, with an OD of 0.54. The lowest removal was observed at 1% concentration with 1-minute contact time, where the OD was 0.13. The 2% concentration of sodium hypochlorite at time of contact of 1 min demonstrated the better activity showing OD of 0.19 than of 1% concentration at 5 minute of contact time resulting the OD of 0.18. Isolate P6 showed the maximum biofilm removal at the 4% concentration after 5 minutes, with an OD of 0.36. The least biofilm removal was noted at 1% concentration after 1 minute, with an OD of 0.09. The activity of the 2% concentration of sodium hypochlorite at time of contact of 1 min showed the activity resulting OD of 0.23 against 1% concentration at 5 minute of contact time resulting the OD of 0.18. Also, the efficiency of 4% concentration of sodium hypochlorite at contact time of 1 min is like 2% concentration of sodium hypochlorite at 5 minute of contact time resulting.

For nearly all isolates of *Pseudomonas aeruginosa* the biofilm removal increased with higher concentrations of sodium hypochlorite and longer contact times. The 4% concentration at 5 minutes generally demonstrated the most effective biofilm removal across all isolates, while the 1% concentration at 1 minute resulted in the least removal. In some cases, the efficiency of removal of biofilm is demonstrated more higher concentration but the time of contact decreases drastically.

In the study 14 isolates were showing the production of biofilm by *Staphylococcus aureus* (Fig 5-7). Isolate S1 shows that the maximum removal of biofilm demonstrated by the 4% concentration for the contact time of 5 minutes, with an optical density (OD) of 0.56. Notably, for this isolate, the 4% concentration also showed removal at 1

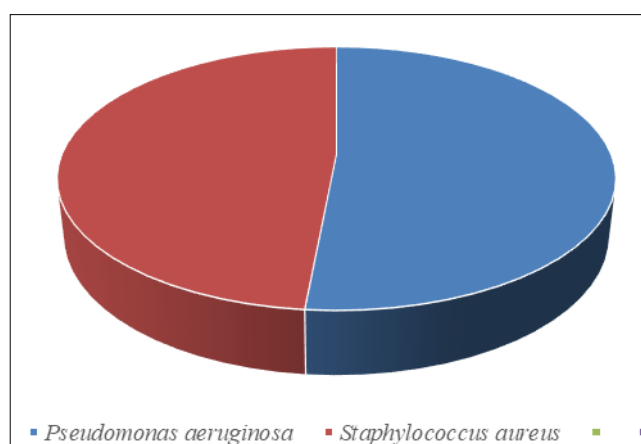
minute (OD = 0.45), though less effective. The minimum removal of biofilm was observed for the 1% concentration at 1 minute of contact time, with an OD of 0.13. Isolate S2 exhibited the maximum biofilm removal at the 4% concentration with a contact time of 5 minutes, resulting in an OD of 0.54. At 1% concentration and 1-minute contact time, the minimum biofilm removal was observed, with an OD of 0.13. Isolate S3 demonstrated the highest biofilm removal at 4% concentration with a contact time of 5 minutes, achieving an OD of 1.21, indicating significant biofilm reduction. In contrast, the minimum removal occurred at 1% concentration for 1 minute, where the OD was 0.09. Isolate S4 showed the maximum biofilm removal at 4% concentration after 5 minutes, with an OD of 0.94, while the least removal was recorded at 1% concentration and 1 minute, with an OD of 0.06. Isolate S5 demonstrated the greatest biofilm removal at 4% concentration for 5 minutes, with an OD of 1.15. The lowest removal was seen at 1% concentration for 1 minute, where the OD was 0.26. Isolate S6 showed the highest biofilm removal at the 4% concentration for 5 minutes, achieving an OD of 1.52, while the lowest removal was noted at 1% concentration and 1 minute, where the OD was 0.07. Isolate S7 exhibited the maximum biofilm removal at 4% concentration and 5 minutes, with an OD of 0.48, while the least removal was observed at 1% concentration for 1 minute, where the OD was 0.07. Isolate S8 demonstrated the highest removal of biofilm at the 4% concentration after 5 minutes, with an OD of 0.48, while the lowest removal was recorded at 1% concentration for 1 minute, where the OD was 0.15. Isolate S9 showed the maximum biofilm removal at 4% concentration and 5 minutes, with an OD of 1.02, while the least removal was at 1% concentration for 1 minute, with an OD of 0.20.

Isolate S10 demonstrated the highest biofilm removal at 4% concentration with 5 minutes, resulting in an OD of 0.43, while the lowest removal was observed at 1% concentration and 1 minute, where the OD was 0.08. Isolate S11 exhibited the greatest biofilm removal at 4% concentration for 5 minutes, achieving an OD of 1.23, while the minimum removal was recorded at 1% concentration and 1 minute, where the OD was 0.16. Isolate S12 showed the highest biofilm removal at 4% concentration and 5 minutes, with an OD of 0.49, while the least removal was observed at 1% concentration for 1 minute, where the OD was 0.11. Isolate S13 demonstrated the maximum biofilm removal at 4% concentration and 5 minutes, with an OD of 0.65, while the lowest removal occurred at 1% concentration for 1 minute, with an OD of 0.11. Isolate S14 exhibited the highest biofilm removal at 4% concentration for 5 minutes, with an OD of 0.71, while the least removal was observed at 1% concentration for 1 minute, where the OD was 0.79.

In all isolates of the *Staphylococcus aureus* the 4% sodium hypochlorite concentration for 5 minutes contact time demonstrated the maximum biofilm removal, with optical densities ranging from 0.48 to 1.52. The 1% concentration having contact time of 1 minute consistently resulted in the least biofilm removal, with optical densities ranging from 0.06 to 0.79. Thus, higher concentrations and longer contact times generally provided more effective biofilm removal for *Staphylococcus aureus*.

Table 1: Collection of samples

Sr. No.	Collection of Samples
1	City Hospital
2	Sahara Hospital
3	Ozone Hospital
4	Lady Harding

**Fig 1:** Prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from non-clinical samples.**Table 2:** Morphological, cultural, and biochemical characteristics of *P. aeruginosa* and *S. aureus*

Sr. No.	Characteristics	Isolates	
Morphological characteristic			
1	Gram Character	Gram -ve rod	Gram +ve cocci
2	Motility	Motile	Non-motile
Cultural characteristic			
3	Shape	Irregular	Circular
4	Size	0.8 μm	0.5 μm
5	Color	Greenish/Blue green	Golden yellow/White
6	Opacity	Translucent/Opaque	Opaque
7	Margin	Undulate	Entire
8	Elevation	Flat/Raised	Convex
9	Surface	Muroid/Rough	Smooth
Biochemical characteristic			
Sugar Fermentation			
10	Glucose A, G	-ve, -ve	+ve, -ve
11	Sucrose A, G	-ve, -ve	+ve, -ve
12	Lactose A, G	-ve, -ve	-ve, -ve
13	Maltose A, G	-ve, -ve	+ve, -ve
IMViC Test			
14	Indole	-ve	-ve
15	Methyl Red	-ve	+ve
16	Voges Proskauer	-ve	+ve
17	Citrate	+ve	-ve
Enzyme Study			
18	Catalase	+ve	+ve
19	Oxidase	+ve	-ve
20	Amylase	-ve	+ve
21	Urease	-ve	+ve
22	Gelatinase	+ve	+ve
23	Coagulase	-ve	+ve
Probable Isolates		<i>P. aeruginosa</i>	<i>S. aureus</i>

Table 3: Screening of biofilm producing *Pseudomonas aeruginosa* and *Staphylococcus aureus* by Tube method.

Sr. No.	Isolates	Total number of isolates	Biofilm Producer	
			Number	Percentage (%)
1	<i>P. aeruginosa</i>	17	6	35.29
2	<i>S. aureus</i>	16	14	87.5

Table 4: Screening of biofilm producing *Pseudomonas aeruginosa* and *Staphylococcus aureus* by Congo Red Agar method.

Sr. No.	Isolates	Total number of isolates	Biofilm Producer	
			Number	Percentage (%)
1	<i>P. aeruginosa</i>	17	6	35.29
2	<i>S. aureus</i>	16	14	87.5

Table 5: Antibiotic Susceptibility test of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Antibiotic	<i>Pseudomonas aeruginosa</i>				<i>Staphylococcus aureus</i>			
	Sensitivity		Resistance		Sensitivity		Resistance	
	No.	%	No.	%	No.	%	No.	%
Ciprofloxacin	17	100	0	0	10	62.5	6	37.5
Gentamicin	17	100	0	0	13	81.25	3	18.75
Erythromycin	3	17.64	14	82.36	4	25	12	75
Ampicillin	0	0	17	100	0	0	16	100
Chloramph-enicol	8	47.06	9	52.94	10	62.5	6	37.5
Amoxiclav	1	5.88	16	94.12	0	0	16	100

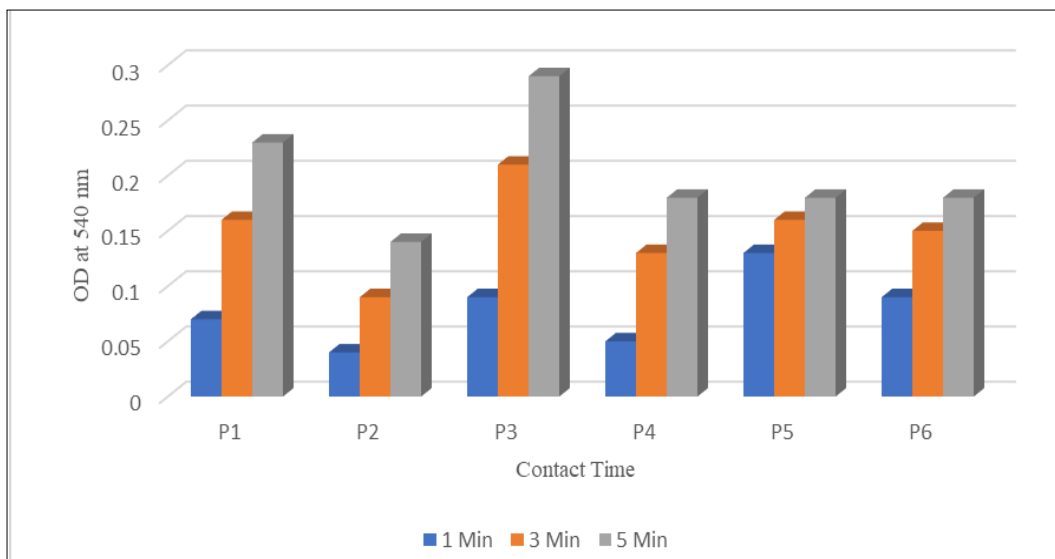


Fig 2: - Effect of 1% Sodium Hypochlorite on *Pseudomonas aeruginosa* at different contact times

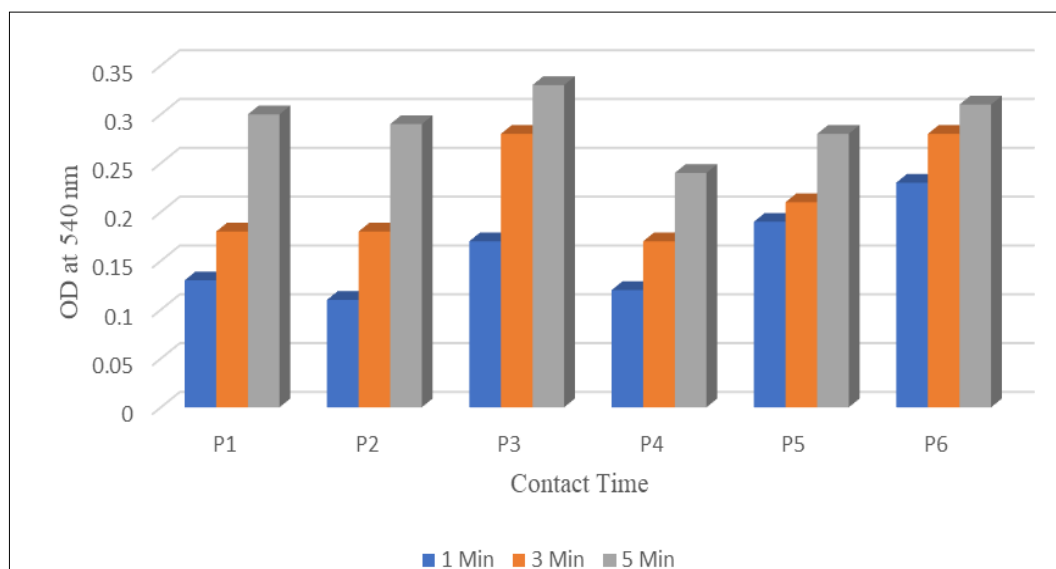


Fig 3: - Effect of 2% Sodium Hypochlorite on *Pseudomonas aeruginosa* at different contact times

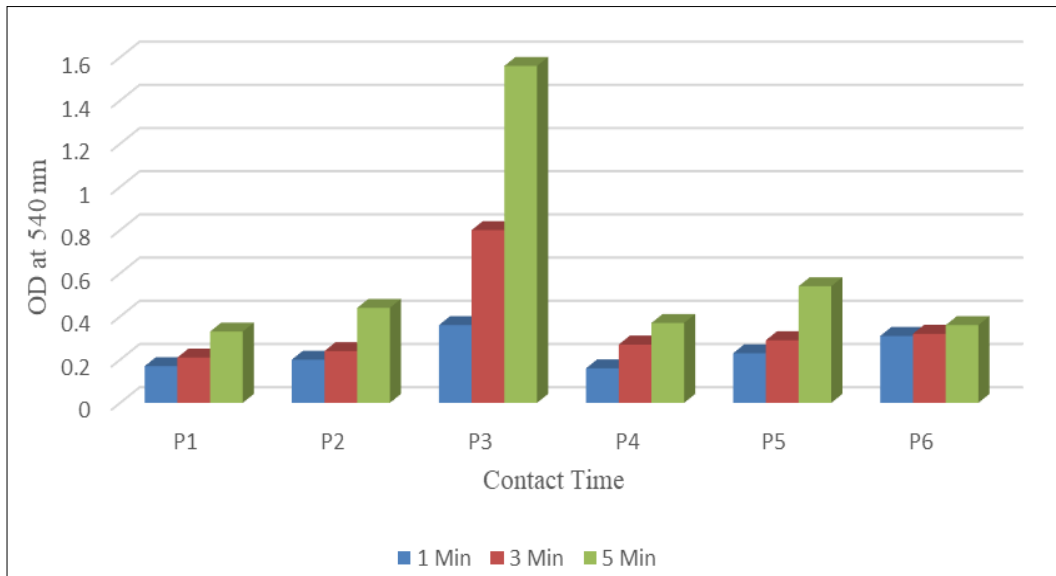


Fig 4: - Effect of 4% Sodium Hypochlorite on *Pseudomonas aeruginosa* at different contact times

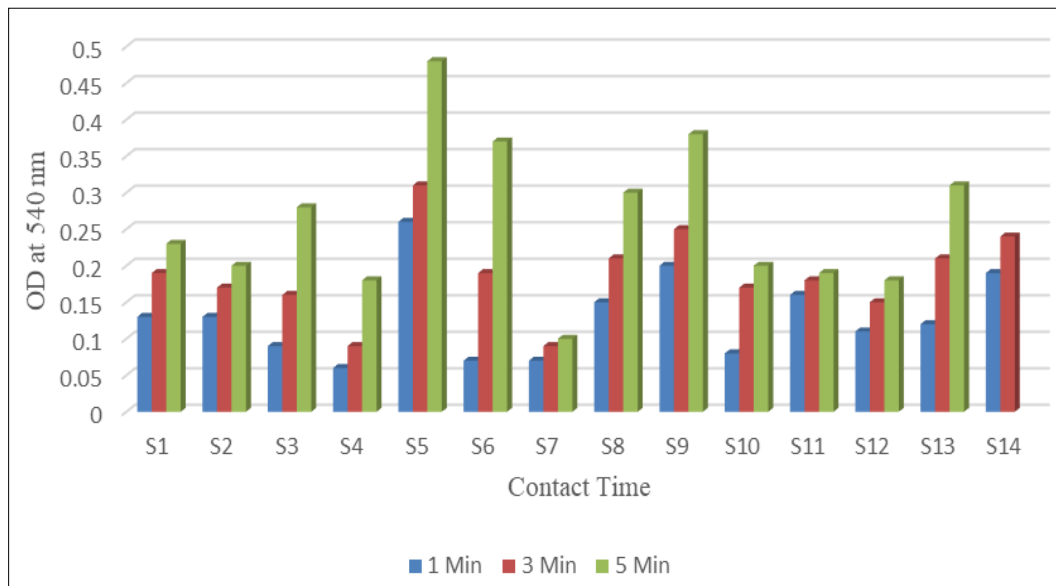


Fig 5: - Effect of 1% Sodium Hypochlorite on *Staphylococcus aureus* at different contact times

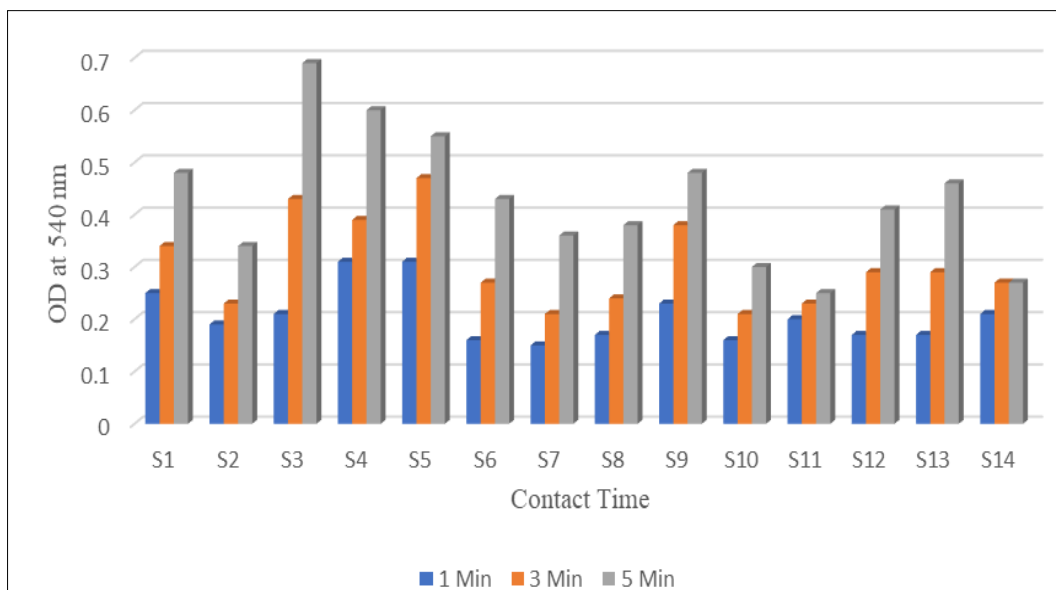


Fig 6: - Effect of 2% Sodium Hypochlorite on *Staphylococcus aureus* at different contact times

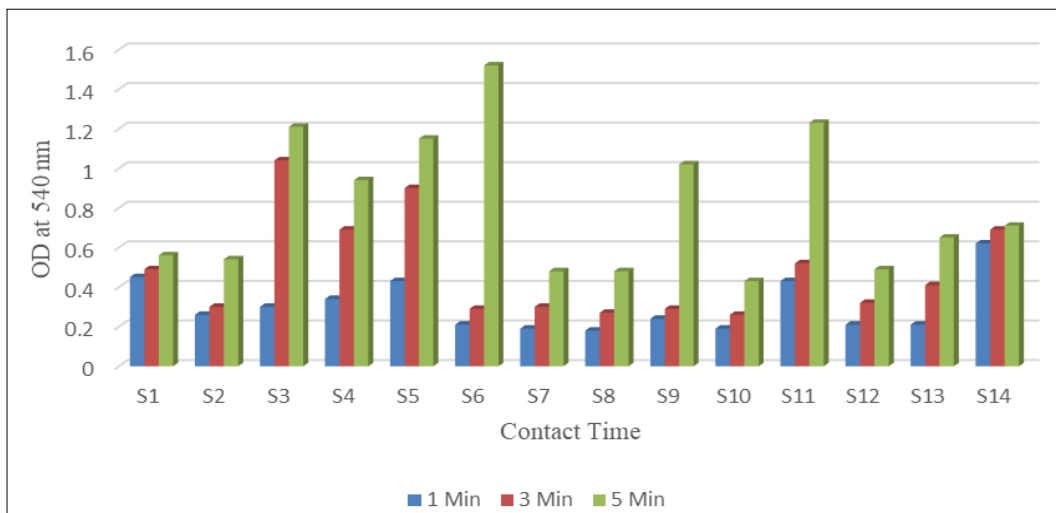
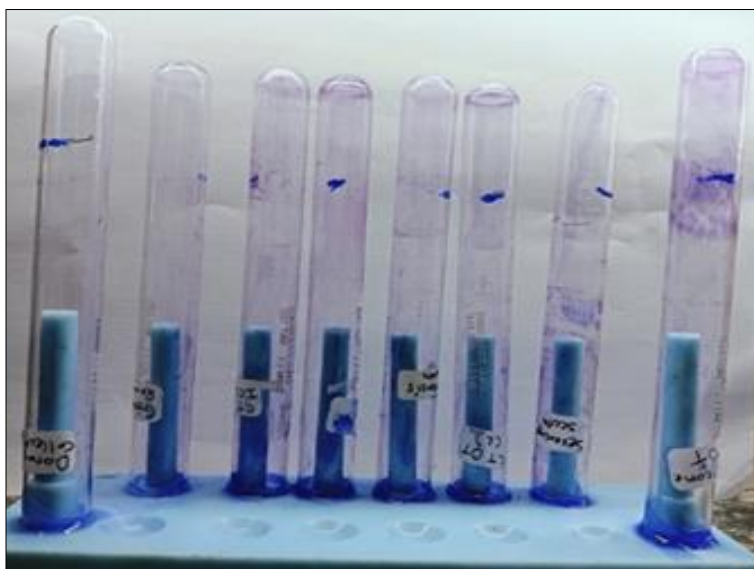


Fig 7: - Effect of 4% Sodium Hypochlorite on *Staphylococcus aureus* at different contact times

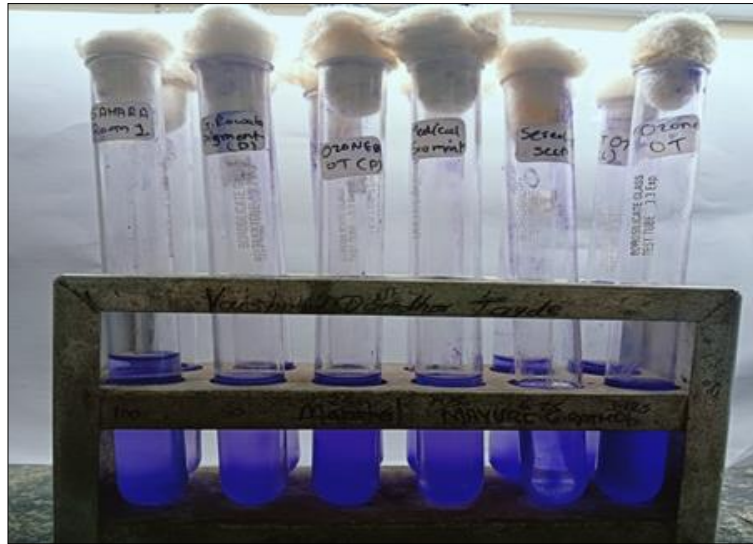
Screening of biofilm producers



1.Tube Method



2. Congo Red Agar Method



Effect of sodium hypochlorite on biofilm removal of isolates

Discussion

The data presented in this study highlights the prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in biofilm formation, with *P. aeruginosa* present in 51.5% of samples and *S. aureus* in 48.4%. In the present study for the detect of biofilm producing bacteria two methods are used as follows: Tube Method (TM) and Congo Red Agar (CRA) method.

In the Tube Method the data shows that out of 17 *Pseudomonas aeruginosa* isolates, 6 (35.2%) were biofilm producers, while out of 16 *Staphylococcus aureus* isolates, 14 (87.5%) were biofilm producers. The results are comparable with the Hassan *et al.*, (2011) [11] showing the prevalence of 49% were showing biofilm formation out of 110 isolates by Tube Method. A study by Li *et al.*, (2021) demonstrated that *P. aeruginosa* isolated from patients with cystic fibrosis showed biofilm formation in approximately 30-40% of the isolates, which aligns findings.

As for the CRA method, the present study indicates that out of 17 *P. aeruginosa* isolates, 6 (35.2%) were biofilm producers while 14 out of 16 *S. aureus* isolates (87.5%) were biofilm producers. Bhattacharya *et al.*, (2018) [6] reported *P. aeruginosa* isolates from patients with chronic infections showing the biofilm production rate which was found to be approximately 40%. Saba *et al.*, (2020) [17] reported *P. aeruginosa* isolates from wound infections, 36% of the *Pseudomonas* isolates were biofilm producers. Alonso *et al.*, (2020) [1] in a broader study on hospital-associated infections, the biofilm showed production rate in *P. aeruginosa* was reported to range from 30% to 50% across different strains.

The Antibiotic Susceptibility Test results for isolates showed that the higher resistance exhibited by isolates to the antibiotic it was supported by others. Bhat *et al.*, (2020) [5] also found that biofilm-forming isolates exhibited significantly higher resistance to antibiotics like gentamicin, ampicillin, and ciprofloxacin than non-biofilm producers. This study concluded that biofilm formation in *P. aeruginosa* contributed to an increased expression of resistance mechanisms such as efflux pumps and beta-lactamase production, making these isolates more difficult to treat. Tiwari *et al.*, (2018) [20] also reported similar observations in a study of biofilm-forming *S. aureus* isolates, which showed a higher resistance to erythromycin, ampicillin, and gentamicin compared to non-biofilm

producers. They suggested that biofilm formation leads to a reduced antibiotic penetration and altered bacterial metabolism, both of which contribute to the enhanced resistance observed in biofilm-producing strains.

In the present study nearly, all isolates of *Pseudomonas aeruginosa* the biofilm removal increased with higher concentrations of sodium hypochlorite and longer contact times. The 4% concentration at 5 minutes generally demonstrated the most effective biofilm removal across all isolates, while the 1% concentration at 1 minute resulted in the least removal. In some cases, the efficiency of removal of biofilm is demonstrated more higher concentration but the time of contact decrease drastically. This in accordance with Hassan *et al.*, (2015) [12] who have reported sodium hypochlorite was found to be highly effective at concentrations of 2.5-5%. They observed that higher concentrations of sodium hypochlorite resulted in more effective biofilm disruption. They reported that a concentration of 5% for a contact time of 10 minutes achieved the best biofilm removal from both gram-negative and gram-positive bacteria, including *Pseudomonas aeruginosa*. This supports present observation that higher concentrations lead to better biofilm removal. Contact time, however, was also found to be a critical factor, with longer exposure resulting in more complete biofilm disruption.

In another study by Sharma *et al.*, (2018) [18], found that sodium hypochlorite at 3-4% concentrations was highly effective in biofilm removal when used for 5-10 minutes. This finding supports the present study, where 4% sodium hypochlorite at 5 minutes was the most effective. They also noted that time played a larger role than the concentration after reaching a threshold concentration of 3%. In their study, contact times of 10 minutes or longer were shown to result in significantly higher biofilm removal rates, particularly when the concentration exceeded 3%.

In the present study the biofilm removal by Sodium hypochlorite on *S. aureus* isolates also showed that at the higher concentration the biofilm removal was prominent and also decreases the time of contact. This is in accordance with the other studied who have also reported the biofilm removal was increased with concentration and decreased time of contact. (Hassan *et al.*, 2015; Sharma *et al.*, 2018; Zong *et al.*, 2019; Bhanduri *et al.*, 2020; Sulaiman *et al.*, 2018) [12, 19, 21].

Conclusion

In the present study it was found that *P. aeruginosa* and *S. aureus* showed prevalence of 51.55% and 48.45% respectively while isolates showed 35.29% and 87.5% production of biofilm by both Tube Method and Congo Red Method. In the biofilm production a high rate of antibiotic resistance was recorded. The study of Sodium hypochlorite reveals that the 4% concentration of Sodium hypochlorite was most effective for the removal of biofilm produced by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It was followed by the 2% and 1% concentration of hypochlorite. The contact time of 5 min shows the maximum removal of biofilm produced following 3 min and 1 min. The concentration of 4% of sodium hypochlorite at 5 min of contact time shows the maximum removal of biofilm and 1% concentration at 1 min contact time shows minimum removal of biofilm.

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