

## Analysis of anti-microbial and anti-biofilm activity of hand washes and sanitizers against *S. aureus* and *P. aeruginosa*

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### Abstract

**Objectives:** To analyse the biofilm-forming potential of clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and to assess antimicrobial activity of commonly used sanitizers in hospital and laboratory settings.

**Method:** The study was conducted at Aga Khan University Karachi from August 2016 to January 2017. The biofilm-forming potential of *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical isolates were evaluated qualitatively using air-liquid interface tube method, and air-liquid interface cover slip assay. The antimicrobial activity of commonly-used hand-washes and sanitizers were assessed using agar well diffusion method, while the anti-biofilm activity of the hand-washes and sanitizers was qualitatively assessed using air-liquid interface cover slip assay.

**Results:** Of the eight hand-washes and sanitizers, 2(25%) showed antimicrobial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while 2(25%) exhibited antimicrobial activity against either *S. aureus* or *P. aeruginosa*. Also, 4 (50%) of them showed no inhibitory activity against *S. aureus* and *P. aeruginosa*.

**Conclusion:** The findings shall have important consequences with regards to infection control in hospital and laboratory settings.

**Keywords:** *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Biofilm inhibition, Hand-washes and sanitizers. (JPMA 70: 100; 2020). <https://doi.org/10.5455/JPMA.2776>

### Introduction

Biofilms have a highly complex architecture of extracellular polysaccharides. The initial step in biofilm formation involves attachment of freely swimming bacteria to an abiotic surface.<sup>1</sup> The attachment of planktonic cells leads to multiplication and multi-layered colony formation within polysaccharides which are secreted by the bacterial species.<sup>2</sup> The multi-layered architecture thickens with time, and, when disrupted, it results in the seeding of planktonic forms.<sup>3</sup> Bacterial biofilms are highly resistant to antimicrobial compounds, disinfectants and antiseptics.<sup>4</sup> Failure of disinfectants to eradicate the biofilm-forming pathogens in hospital settings results in a huge burden of nosocomial infections as well as the development of antimicrobial resistance.<sup>5</sup> Although the use of surface disinfections remain a routine practice in hospital settings to prevent 'bio-contamination' of the surfaces and for hand hygiene, biofilm-protected bacteria shows strong

resistance against such "biocidal" treatment.<sup>6</sup>

The efficacy of commercial disinfectants or biocides have been studied,<sup>7</sup> but there is limited research available related to the efficacy of disinfectants against biofilm-forming pathogens in the clinical settings.<sup>8</sup> Clinical isolates of *Pseudomonas (P.) aeruginosa* have been found to be causing both community-acquired infections (CAIs) as well as nosocomial, especially in patients with history of intravenous (IV) drug use-associated infections, burn wounds, acute leukaemia, cystic fibrosis, organ transplants, corneal infections.<sup>9</sup> Increase in the prevalence of vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin-resistant *Staphylococcus (S.) aureus* (MRSA) calls for innovative methods to control the dissemination of the nosocomial infections.<sup>10,11</sup>

The current study was planned to analyse the biofilm-forming potential of clinical isolates of *P. aeruginosa* and *S. aureus*, and to assess antimicrobial activity of commonly used hand-washes and sanitizers in hospital and laboratory settings.

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## Material and Methods

This study was conducted at Aga Khan University Karachi from August 2016 to January 2017 and comprised clinical isolates of *S. aureus* and *P. aeruginosa* that were procured from a diagnostic microbiological laboratory. Qualitative determination of biofilm formation by *S. aureus* and *P. aeruginosa* was done.

The biofilm-inhibition potential of commonly used hand-washes / sanitizers against *S. aureus* and *P. aeruginosa* isolates were evaluated by air-liquid interface tube method, and air-liquid interface cover slip assay. In brief, 3-5ml of Tryptone Soy Broth (Sigma-Aldrich) containing tubes were inoculated with *S. aureus* and *P. aeruginosa* and were allowed to grow over 48 hours at 37°C.<sup>12</sup> Thereafter, the dense matt of the biofilm was registered at the junction of air and liquid interphase inside the tubes.

By utilizing the air liquid interface cover slip assay, the *P. aeruginosa* and *S. aureus* biofilms were observed under the light microscope.<sup>13</sup> Briefly, 3ml TSB (Sigma Aldrich) was poured into flat bottom plates of the 12-wells with the help of pipettes. Subsequently, 300µL of *S. aureus* and *P. aeruginosa* cultures were added to the broth, followed by a 90° angle placement of sterile cover slips with against the surface of the well. All of the 12 well plates allowed sitting in a static manner and incubating at 37°C for the period of 48h. Thereafter, 0.1% w/v crystal violet was poured over the cover slips for 15 minutes, followed by washing with the distilled sterile water. It was followed by washing and drying of the cover slips. These dried cover slips observed with a light microscope (Olympus, Japan) and the images were obtained at a 40X magnification with the help of the microscope mounted visualization camera.

The antimicrobial activity of commonly used hand-washes and sanitizers were assessed using agar well diffusion method. Briefly, a bacterial lawn of *S. aureus* and *P. aeruginosa* was prepared on the nutrient agar plates by placing 1ml of the culture broth of each pathogen onto the nutrient agar plate and the culture was spread on the plate with a capillary glass lawn maker. Approximately, 5mm deep wells were burrowed inside the agar nutrient plate.

Subsequently, 150µL of each inhibitor was poured into the burrowed well, and allowed to incubate at 37°C for a period of 24 hours. Afterwards, the zones of the

inhibition were visualized and measurement in millimetres was undertaken around the burrowed wells.<sup>14</sup>

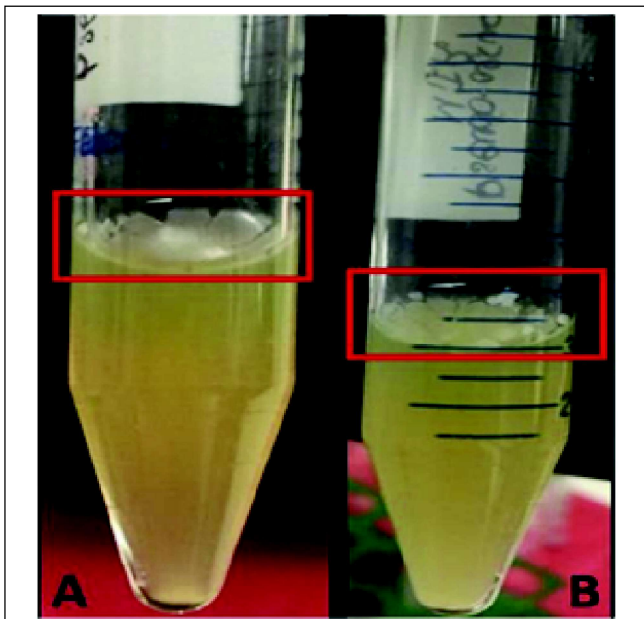
The anti-biofilm activity of commonly used hand-washes and sanitizers were qualitatively assessed using air-liquid interface cover slip assay. Briefly, 3ml TSB (Sigma Aldrich) was pipetted into 9 wells of two different 12-well, flat-bottom plates. Subsequently, 300µL of *P. aeruginosa* was added to each well of one plate and addition of 300µL of *S. aureus* culture was done to each well of another plate. The incubation of these plates were allowed at 37°C for 3 hours. Thereafter, 150µl of each inhibitor was added to each well of both plates, while one well per plate served as control. Subsequently, in each well sterile cover slips were placed at 90° of angle against the well's surface. These well plates containing 12 wells were incubated at 37°C for 48 hours. Thereafter, 0.1% w/v crystal violet was poured over the taken out cover slips for 15 minutes, followed by washing with the distilled sterile water. Then washing of these cover slips was undertaken followed by drying. Again, these dried cover slips observed with a light microscope (Olympus, Japan) and the images were obtained at a 40X magnification with the help of the microscope mounted visualization camera.

## Results

For the tube method, the naked eye visual examination of the tubes revealed dense whitish matt, which is a tell-tale sign of the biofilm formation by *S. aureus* and *P. aeruginosa* (Figure 1). Similarly, a dense biofilm formation as well as a stronger cellular aggregation was observed related to cultures of *S. aureus* and *P. aeruginosa* on the air liquid assay. (Figure 2A-B).

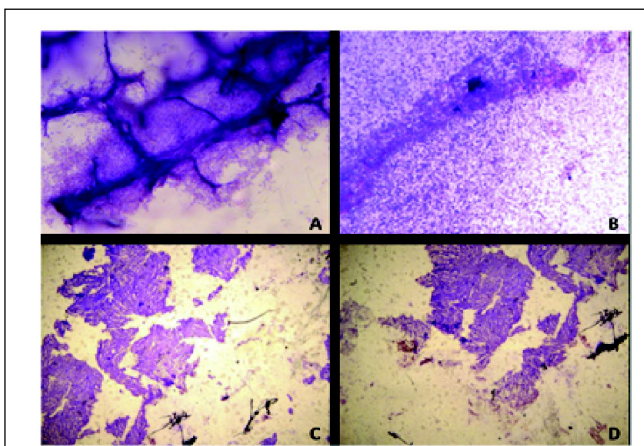
Of the 8 commonly used hand-washes and sanitizers, only 2(25%) (Table; inhibitors titled as 2 and 4) showed an antimicrobial activity against both *S. aureus* and *P. aeruginosa*, while 2(25%) exhibited antimicrobial activity against either *P. aeruginosa* (Table; inhibitor 5) or *S. aureus* (Table; inhibitor 7) activity. Four (50%) of them showed no inhibitory activity against both *S. aureus* and *P. aeruginosa* (Table).

In comparison, no agent was able to completely inhibit or remove the biofilm formation by *P. aeruginosa* (Figure 3 A-I) and *S. aureus* (Figure 3 J-R). Only 1(12.5%) inhibitory agent (No. 1) showed some biofilm disruption and inhibition against *S. aureus* biofilm (Figure 3K), while the remaining 7(87.5%) agents did not inhibit the biofilm

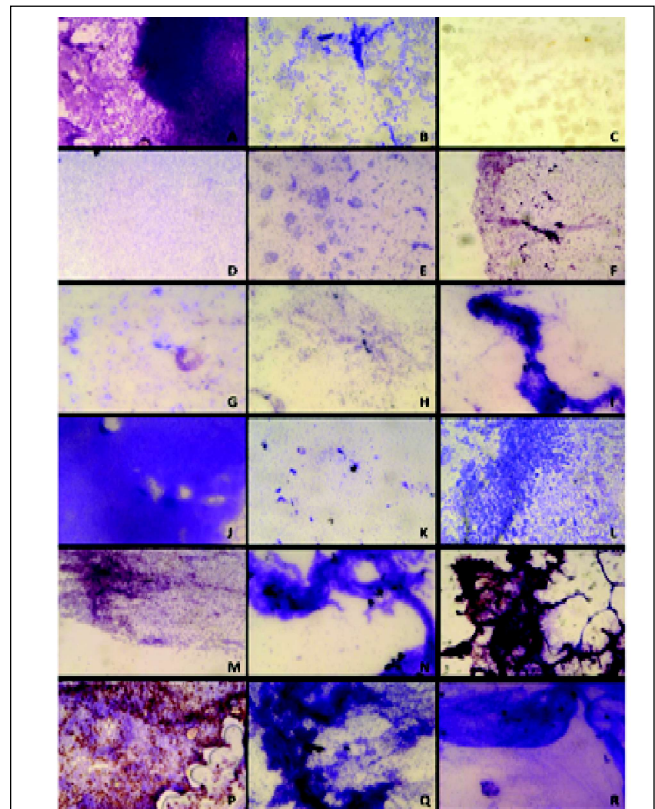


**Figure-1:** Qualitative analysis of biofilm formation by *Pseudomonas aeruginosa* (*P.*) and *Staphylococcus* (*S.*) *aureus* using Air-liquid interface tube method: the images shows the formation of a whitish dense matt at the Air liquid interface in the tube (red box) by A) *P. aeruginosa* and B) *S. aureus*.

formation in which dense matt formation was seen (Figure 3 J-R). In comparison, biofilm formation by *P. aeruginosa* was not inhibited by any of the inhibitory agents tested, except 1(12.5%) inhibitory agent (No. 2) (Figure 3C) which showed some disruption of biofilm formation (Figure 3 A-H).



**Figure-2:** Qualitative analysis of biofilm formation by *Pseudomonas* (*P.*) *aeruginosa* and *Staphylococcus* (*S.*) *aureus* by employing the Air-liquid interface cover slip method: the picture shows the formation of the dense matt along with the microbial aggregation under inverted microscope using Air liquid interface cover slip assay as observed for A and B) *P. aeruginosa* and C and D) *S. aureus*. Crystal violet was used to stain the microbial cells and the biofilms.



**Figure-3:** Qualitative analysis of anti-biofilm activity of eight inhibitors assessed by Air-liquid interface cover slip method: the picture shows the formation of the dense matt along with the microbial aggregation (biofilm formation) observed under inverted microscope by A-I) *Pseudomonas* (*P.*) *aeruginosa* and J-R) *Staphylococcus* (*S.*) *aureus* after addition of B and K) inhibitor 1, C and L) inhibitor 2, D and M) inhibitor 3, E and N) inhibitor 4, F and O) inhibitor 5, G and P) inhibitor 6, H and Q) inhibitor 7, and I and R) inhibitor 8. Pictures A and J shows biofilm formation in control wells by *P. aeruginosa* and *S. aureus*, respectively. Crystal violet was used to stain the microbial cells and the biofilms.

### Discussion

The findings showed that half of the 8 inhibitory agents tested had some antimicrobial activity against the clinical isolates of *P. aeruginosa* and *S. aureus* (Table). This was important as seven out of eight claimed to be more than 99.9% effective in killing off the bacteria.

Three of our inhibitory products contained triclocarban (TCC) which is a commonly used antiseptic ingredient with broad-spectrum antimicrobial effect and it is frequently utilized as personal hygiene articles, i.e. soaps and sanitizers.<sup>15</sup> A great majority (>84%) of antimicrobial soaps contain TCC as an active ingredient.<sup>16</sup> The lack of inhibition of our tested pathogens is profound since they were incubated over 24 hours, giving them enough

**Table:** List of inhibitory agents tested for their antimicrobial activity against *Pseudomonas (P.) aeruginosa* and *Staphylococcus (S.) aureus*: The table enlists eight inhibitory agents tested in this study, along with active agent present in each inhibitor and antimicrobial activity claimed by each product. The table also shows zone of inhibition observed for each agent when tested against *P. aeruginosa* and *S. aureus*.

| Inhibitor | Product claim | Active agents   | Zone of inhibition (mm)       |                              |
|-----------|---------------|---|-------------------------------|------------------------------|
|           |               |   | <i>Pseudomonas aeruginosa</i> | <i>Staphylococcus aureus</i> |
| 1         | Kills 100%    | Triclocarban (TCC)  | 0                             | 0                            |
| 2         | Kills 99.99%  | Triclocarban (TCC)  | 20                            | 14                           |
| 3         | Kills 99.9%   | Salicylic Acid  | 25                            | 20                           |
| 4         | Kills 99%     | Triclocarban (TCC)  | 0                             | 0                            |
| 5         | Kills 99.9%   | Alcohol IP (Denatured) eq. to absolute alcohol 72.34% v/v         | 16                            | 0                            |
| 6         | Kills 99.99%  | Isopropyl Alcohol IP 10% w/w and Ethyl Alcohol 95% V/V IP 55% w/w | 0                             | 0                            |
| 7         | N/A           | Ethyl Alcohol and Isopropyl Alcohol                               | 0                             | 11                           |
| 8         | Kills 99.9%   | Ethyl Alcohol   | 0                             | 0                            |

time to act against the pathogens compared with the short time span required for hand-washing with soaps or sanitizers. The study found that one agent which contained TCC did show some disruption of the biofilm formed by *P. aeruginosa*, but failed to completely remove or inhibit the biofilm formation by either *P. aeruginosa* or *S. aureus*. Alcohol-based preparations have been shown to have antimicrobial activity,<sup>17</sup> however, we did not find any anti-biofilm activity in any of the inhibitors except one which we used against the two isolates. Interestingly, no hand-wash and sanitizer was effective in inhibiting and removing the biofilm formation by *S. aureus* and *P. aeruginosa* clinical isolates. This has important consequences with regards to infection control in hospital and laboratory settings. These hand-washes and sanitizers give false sense of security to the clinicians and lab researchers alike and may be considered an important dimension of infection control in the clinical and research laboratory settings. The limitations of the current study are its small sample size and insufficient quantitative analysis of the results, which could be addressed by adding more bacterial strains and designing and carrying out more specific and detailed investigations.

## Conclusion

The findings have important consequences with regards to infection control in hospital and laboratory settings. Such analyses may be useful in monitoring the efficacy of the commonly used disinfectants, which is an essential step in devising effective infection control strategies.

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**Conflict of interest:** None.

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