Comparative analysis of novel suspension testing and agar cup diffusion methods in establishing the susceptibility of *Pseudomonas aeruginosa* against ethanol and chlorhexidine gluconate

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**ABSTRACT**

*Pseudomonas aeruginosa* is one among the leading nosocomial pathogens worldwide. It is therefore necessary to decrease and to prevent a rebound of growth. Comparison of novel suspension testing method and agar cup diffusion method results in determination of the sensitive method to identify effectiveness of disinfectants against microbial activity. This study was carried out to determine the effectiveness among novel suspension testing and agar cup diffusion method to determine disinfectant susceptibility and also to identify the efficacy of ethanol and chlorhexidine gluconate at manufacturer’s concentration against *Pseudomonas aeruginosa*. In this study 50 isolates of *Pseudomonas aeruginosa* were included. Each isolate was subjected to novel suspension testing method and agar cup diffusion method with ethanol and chlorhexidine gluconate, the results were observed and recorded. The 50 isolates, sensitive strains showed 100% sensitivity to chlorhexidine gluconate and 95% to ethanol. Whereas resistant strains showed 100% sensitivity to chlorhexidine gluconate, 75% were sensitive to ethanol. Both agar cup diffusion method and novel suspension method yielded similar results. With the advantage of easy processing and less time consumption, agar cup diffusion method can be routinely used for determining the disinfectant susceptibility testing.

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**INTRODUCTION**

*Pseudomonas aeruginosa* is one among the leading nosocomial pathogens worldwide. The infections caused by this organism are often hard to treat because of the intrinsic resistance exhibited by the species to most of the antibiotics. They possess multiple mechanisms of drug resistance. It is not only necessary to decrease a wide spread of resident and transient microbes to sub pathogenic levels and also to prevent the recurrence of growth of this genus (Gluck, 2004a). Disinfectant plays a vital role in reducing the spread of nosocomial infection. It is therefore necessary to perform disinfectant susceptibility testing and identify the efficacy of disinfectants against these isolates (Russell and Day, 1993). However studies show that these organisms produce biofilms and resistance to disinfectants has also been identified. So it becomes important to maintain proper surveillance and management of these organisms (Gunasekar et al., 2018). The testing of disinfectants in a routine laboratory is very important in order to determine their correct concentration of practical usage. The aim of this study
is to identify the better method among novel suspension testing method and agar cup diffusion method to compare and to analyze, which is done against Pseudomonas aeruginosa isolates with ethanol and chlorhexidine gluconate. The sensitivity and specificity of both the method can be identified and the better method can be determined, which can be used in laboratory for routine susceptibility testing of disinfectant at manufacturer’s concentration.

MATERIALS AND METHODS

The descriptive study on disinfectant susceptibility testing of Pseudomonas aeruginosa against ethanol and chlorhexidine gluconate was carried out in the Clinical Microbiology Laboratory at Saveetha Medical College and Hospital, Thandalam, Chennai-602105, Tamil Nadu, India, after getting approval from the Institutional Review Board. 50 different strains of Pseudomonas aeruginosa from different clinical specimens received in the Clinical Microbiology Laboratory were included in this study. They were characterized by conventional culture methods and biochemical tests: oxidase test, Triple sugar ion agar testing, Indole, Urease hydrolysis, Citrate utilization and Mannitol motility medium (Costerton and Anwar, 1994). Antibiotic susceptibility testing of the isolates was determined by conventional methods and tabulated (CLSI, 2015). In this examination two skin disinfectants namely ethanol and chlorhexidine gluconate (Gluck, 2004b) at manufacturer’s concentration was inactivated by diluting 10⁻⁰ with specific neutralizers. Neutralizers used were tween 80 for chlorhexidine gluconate and normal saline for ethanol. Then 100μl of each of the solutions was transferred to nutrient agar plates in triplicates in order to reduce error. They were incubated at 37°C for 72 hours. The number of colonies in each plate were counted and tabulated in Table 2. Figure 1 and Figure 2 depicts the procedure. The antimicrobial activity was considered to be inactive if there is a decrease in the colony count to 5% as compared to the control (Alabi and Sanusi, 2012).

Agar Cup Diffusion Test

Agar cup diffusion assay is one of the methods for quantifying the ability of antibiotics to inhibit bacterial growth. The disinfectant is allowed to diffuse freely in the solid nutrient medium (Jayakumar, 2011). By agar cup diffusion method each strain of Pseudomonas aeruginosa was subjected to disinfectant susceptibility testing against ethanol and chlorhexidine gluconate at manufacturer’s concentration. 20ml of Mueller Hinton Agar was autoclaved and cooled. Then this molten agar was seeded with 2μl of dilution from an overnight broth culture of the individual strain, mixed and poured into the sterile Petri dishes and allowed to set. The surface of the plate were dried and with the aid of a sterile 8mm cup borer, four wells were bored in the agar plate, the first well was filled with 10 μl of chlorhexidine gluconate, the second well was filled with 10 μl of ethanol, the third well served as the positive control, which is being placed with colistin drug disc and the final well was filled with normal saline which was the negative control.

This whole procedure was done in duplicates. The plates after one hour of pre-diffusion were then incubated at 37°C for 24 hours in an inverted position. The average of the zones of growth inhibition were then recorded and tabulated in Table 3 and shown in Figure 3.

RESULTS AND DISCUSSION

In this study 50 isolates of Pseudomonas aeruginosa were subjected to disinfectant susceptibility testing. The results obtained are tabulated. In both the novel suspension testing method and agar cup diffusion method, isolates shown sensitive to aminoglycosides, fluoroquinolone, cephalosporin and carbapenam strain showed 100% sensitivity
Table 1: Antibiotic susceptibility testing of *Pseudomonas aeruginosa* isolates.

| S.no | Antibiotics       | Sensitive % | Resistant % |
|------|-------------------|-------------|-------------|
| 1.   | Amikacin          | 19          | 31          |
| 2.   | Ciprofloxacin     | 18          | 32          |
| 3.   | Ceftazidine       | 20          | 30          |
| 4.   | Cefaperazone sulbactm | 10    | 40          |
| 5.   | Imipenam          | 21          | 29          |
| 6.   | Meropenam         | 21          | 29          |

Table 2: Disinfectant susceptibility testing of *Pseudomonas aeruginosa* by novel suspension testing method.

| *Pseudomonas aeruginosa* | Chlorhexidine gluconate | Ethanol |
|--------------------------|-------------------------|---------|
|                          | 0.5% | 0.25% | 0.125% | 99% | 48.5% | 24.25% |
| Sensitive strain          | 2%   | 0%    | 0%     | 22% | 13%   | 9%     |
| *MDR strain*              | 1.5% | 0%    | 0%     | 12% | 8%    | 5%     |

(*MDR strain – multidrug resistant strain)

Table 3: Disinfectant susceptibility testing of *Pseudomonas aeruginosa* by agar cup diffusion method.

| *Pseudomonas aeruginosa* | Chlorhexidine gluconate | Ethanol |
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(*MDR strain – multidrug resistant strain)

Figure 1: Novel suspension testing of *Pseudomonas aeruginosa* with chlorhexidine gluconate. A1 - Growth observed at 15 sec, A2 - observation at 30 sec, A3 - Observation at 60 sec, A4 - control
Figure 2: Novel suspension testing of *Pseudomonas aeruginosa* with ethanol. B1 - growth observed at 15 sec, B2 - observation at 30 sec, B3 - observation at 60 sec, B4 - control

**Pseudomonas aeruginosa**

Figure 3: Agar cup diffusion method of *Pseudomonas aeruginosa* with chlorhexidine gluconate and ethanol.

Effective skin antiseptics are needed in preventing the increased incidence of infection during patient care. *Pseudomonas aeruginosa* being one of the most important microorganisms responsible for four categories of Hospital-acquired infections (HAI). This can however be reduced at the point of occurrence by means of proper personal protections. Skin disinfectants play a vital role in preventing the occurrence of such infection. Therefore it is necessary to use proper disinfectant. This can help reduce the use of third-line drugs which may lead to nephrotoxicity. Thus, by next decade hospitals should be made free of nosocomial infections. Hope, this in turn helps to increase the standard of living in India.

**CONCLUSIONS**

Effective skin antiseptics are needed in preventing the increased incidence of infection during patient care. *Pseudomonas aeruginosa* being one of the most important microorganisms responsible for four categories of Hospital-acquired infections (HAI). This can however be reduced at the point of occurrence by means of proper personal protections. Skin disinfectants play a vital role in preventing the occurrence of such infection. Therefore it is necessary to use proper disinfectant. This can help reduce the use of third-line drugs which may lead to nephrotoxicity. Thus, by next decade hospitals should be made free of nosocomial infections. Hope, this in turn helps to increase the standard of living in India.

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In this study *Pseudomonas aeruginosa* isolates showed complete susceptibility to chlorhexidine gluconate and intermediate susceptibility to ethanol. Hence it is better to use hand washes with chlorhexidine gluconate at manufacturer’s concentration whereas ethanol can be used in hand rubs. In the study conducted by Gunasekar *et al.* (2018) 100% of isolates were susceptible to chlorhexidine gluconate at manufacturer’s concentration. But when the dilution was made half to the manufacturer’s formulation, 4% resistance was observed. Likewise, 8% of resistance was observed when it was further diluted. In another study conducted by Alabi and Sanusi (2012), some of the clinical isolates exhibited resistance to the disinfectant formulations at the dilution prescribed by the manufacturer. In other study conducted by Jayakumar (2011) chlorhexidine gluconate effectiveness was improved by the addition of 80% ethyl alcohol.
Conflict of Interest
None.

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