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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 con-

centration 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 disinfectants 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 hydrolysation, 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 were subjected to efficacy testing by agar cup diffusion method and novel suspension testing method (Kampf and Kramer, 2004). The susceptibility pattern of organism is shown in Table 1.

Novel Suspension Testing Method

The antimicrobial effectiveness of the disinfectants is directly proportional to the measurement of microbial population reduction at a specific time and point after the exposure to the tested disinfectant (Alabi and Sanusi, 2012).

Preparation of the test organism: *Pseudomonas aeruginosa* strains were inoculated in test tubes containing 5ml of peptone water and kept in incubator at 37°C for overnight incubation. The suspension containing 10^9 Colony Forming Unit per milliliter (0.5 McFarland) was used as the test inoculums.

The suspension testing Procedure

The test inoculums (10μl) were added to 5ml of each of the disinfectant solution. Then it was vortexed for 5 seconds to obtain a bacterial density of $2\times$CFU/ml approximately. The inoculums were added to 5ml of physiological saline and this was used as the control suspension. The inoculums were exposed to the disinfectants for 15, 30, 60 seconds at room temperature. The antimicrobial activity of the disinfectants in the suspensions was inactivated by diluting 10μl of each of the suspensions 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 72hours. 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 sold 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, fluroquinolone, cephalosporin

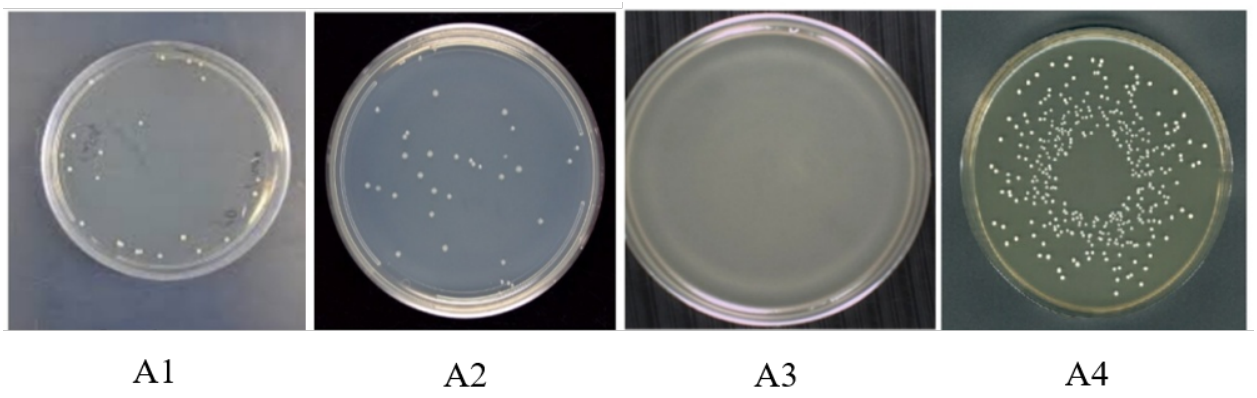

Figure 1: Novel suspension testing of *Pseudomonas aeruginosa* with chlorhexidine gluconate. A1-Growth observed at15 sec, A2 - observation at 30 sec, A3 -Observation at 60 sec. A4 - control

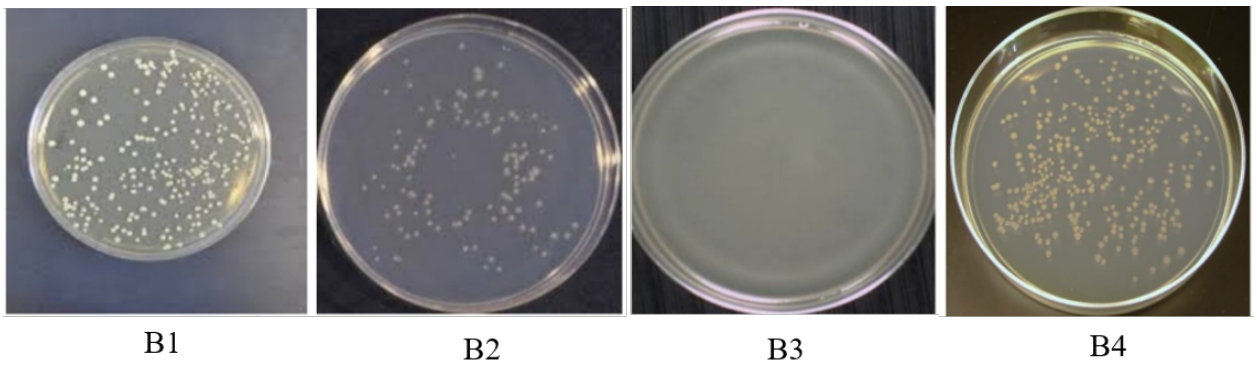

Figure 2: Novel suspension testing of *Pseudomonas aeruginosa* with ethanol. B1 - growth observedat 15 sec, B2- observation at 30 sec, B3 - observation at 60sec, B4 – control

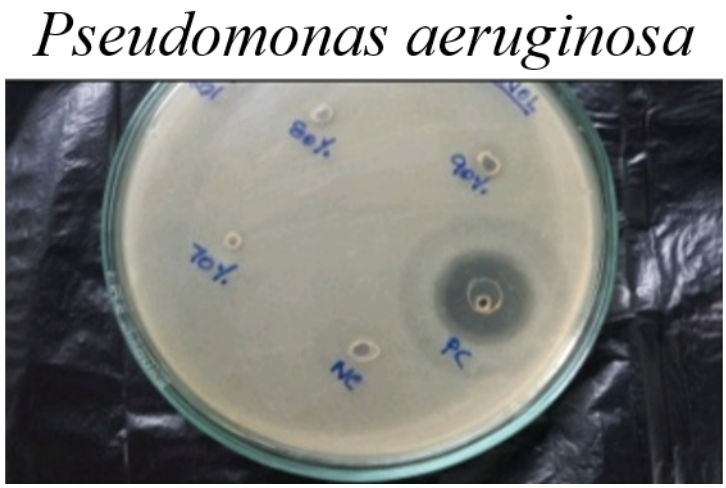

Figure 3: Agar cup diffusion method of *Pseudomonas aeruginosa* with chlorhexidine gluconate and ethanol

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 suscepiblity testing of *Pseudomonas aeruginosa* by agar cup diffusion method

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and carbapenam strain showed 100% sensitivity to chlorhexidine gluconate at manufactures concentration, whereas carbapenam and ESBL resistant strains showed only 75% sensitivity to ethanol. The results obtained by both the methods were similar. With the advantage of less time consumption, less materials and easy processing agar cup diffusion method is preferred. 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.

CONCLUSION

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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Conflict of Interest

None.

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