IJBPAS, April, 2015, 4(4): 1989-1999

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DETECTION OF THE MOST EFFECTIVE DISINFECTANT AGAINST MDR BACTERIA IN HOSPITAL INFECTIONS

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ABSTRACT

Worldwide emergence of multi-drug resistant organism remains major problem in causing nosocomial infection. In addition to which there is an emergence of resistance to chemical disinfectants as well. Hence prevention of health care associated infection has become an alarming concern and challenge for clinicians. This can be achieved by proper infection control practices, appropriate use of sterilization and disinfection procedure.

Bacteria isolated from different clinical specimens of hospitalized patients were identified, among which the most resistant strains were detected by standard protocol 'Kirby-Bauer disc diffusion method'. These isolates were subjected for bactericidal activity testing against commonly used disinfectants such as Phenol, Chlorhexidine gluconate (CHX), Benzylkonium Chloride (BZT), Chloroxylenol and Sodium hypochlorite. Efficacies of these disinfectants were evaluated by Minimum inhibitory concentration method and Time kill assay.

Among the five disinfectants, Benzylkonium Chloride (BZT), Chloroxylenol showed the better activity for both Gram positive and Gram negative organisms, followed by chlorhexidine gluconate and sodium hypochlorite. Phenol was found to be ineffective to all the isolates.

The effect of disinfectants for Gram positive and Gram negative organisms were varied according to the environmental condition and exposure of time. Hence in the hospital

environment it is necessary to choose the appropriate disinfectant in infection control practices to avoid cross contamination and nosocomial outbreak.

Keywords: Disinfectants, Antibacterial Efficacy, Minimum inhibitory concentration, Multi-drug Resistance, Time Kill Assay

INTRODUCTION

The increasing emergence and spread of multi-drug resistance (MDR) bacteria in hospitals continues to be a challenge for infection control practices worldwide. In spite of all efforts made to improve hospital hygiene, nosocomial infections still remains extensive risk to patients and added burden to hospitals **[1]**. Sterilization and disinfection go hand in hand, in the prevention of disease spread and development of Multi-drug resistant strains in hospitalized patients. Failure of this can result in many hospital-acquired infections thus leading to increased morbidity and mortality **[2]**.

The rise in MDR strains in the hospital is due to the antibiotic abuse and over the counter drug dispensing. It has become a huge problem for treating such MDR infection. These problems cannot be controlled on day to day basis and on all levels. One of the methods in the prevention of spread of MDR microbes in the healthcare set up is proper use of disinfectant. So it is vital importance in infection control practices **[3]**.

Utilization of phenolic constituent of some phenolic disinfectant as carbon source by some bacteria such as *Pseudomonas aeruginosa* and complete resistance to some microorganisms to some of the classes of disinfectants eg. *Staphylococococcus aureus* have also been previously reported **[4]**. The resistance of pathogens to disinfectant is an emerging problem and is due to presence of integrons, super integrons and efflux mechanisms in the pathogen **[5]**. But there is only limited awareness among health care workers about choosing an appropriate disinfectant, especially in small healthcare settings. Usually, an agent with broad-spectrum antimicrobial activity is chosen based on the literature provided by manufacturers **[6]**. The effect of various disinfectants on MDR isolates varies with type of disinfectant, bacterial concentration, presence or absence of organic matter and other pollutant along with the time of exposure to bacterial isolates.

Hence this research paper was designed to study the efficacy of locally available disinfectants, which are mainly used for disinfection of surfaces, infectious microbial and other hospital wastes. Therefore this

study could create awareness not only to the healthcare worker in choosing appropriate disinfectants but also in preventing cross transmission of MDR strains with hospital environment.

MATERIALS AND METHODS

Bacterial Isolates

A total of 25 clinical samples were collected from Shri SathyaSai Medical College and Research Institute, Thiruporur, Department of Microbiology. The bacterial strains were identified as per the standard protocol **[7]**. Out of 25 samples, 10 MDR strains such as Methicillin resistance *Staphylococcus aureus*, Vancomycin Resistant Enterococci, ESBL producing *Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Enterobacter aerogenes*, *Proteus mirabilus*, *Proteus vulgaris*, MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were taken for this study.

Disinfectants

Phenol, Chlorhexidine gluconate (CHX), Benzylkonium Chloride (BZT), Chloroxylenol and Sodium hypochlorite were used for the study.

Minimum Inhibitory Concentration:

Minimum inhibitory concentration of the disinfectants was performed in two fold dilution by agar dilution method according to the CLSI guidelines **[8]**. The dilution of MIC ranges from 1:20 to1:1280. Overnight culture suspensions of bacterial isolates were adjusted to 0.5 McFarland standard for turbidity and 10 µL of suspension was spot inoculated onto Muller Hinton agar plates with above mentioned concentrations of disinfectants. ATCC strains of *Escherichia coli* 25922, *Staphylococcus* aureus 25923 were used as control strains.Inoculated plates were incubated at 37°C for 24 hours and lowest concentration of disinfectants that inhibited the growth of bacterial isolates was considered as MIC **[3]**.

Time Kill Assay

The experiment was carried out as per standard protocol **[9]**. By using five different disinfectants mentioned above. For each tube, 0.1ml of culture solution was added into 0.9ml of disinfectant at different contact time: 30sec, 1min, 5min, 10min, 30 min, 60 min, and 120minrespectively. After certain contact time, tubes were centrifuged at 5000rpm for 5min to separate the culture from the solution. Supernatant was discarded and then the tube was refilled by deionized water, 50µL aliquots of the mixture were spread on the plate and incubated at 37°C for 24hrs. The next day, number of colonies on the plate was counted and cell survival rates were calculated.

RESULT

A total of 10 MDR strains were included in this study. All the isolates were screened for antimicrobial resistance pattern by following Kirby Bauer disc diffusion technique with the following antibiotics.

Oxacillin-OX (1mcg), Ampicillin-AMP (10mcg), Amikacin-AK (30mcg), Gentamicin-GEN (10mcg), High Level Gentamicin- HLG (120mcg), Ceftazidime-CAZ (30mcg), Cefazolin- CZ(30mcg), Cefotaxime-CTX(30mcg), Cefepime-CPM(30mcg), Imipenem-IMP (10mcg), Ciprofloxacin-CIP(5mcg), Erythromycin-ERY (15mcg), Tetracyline–TE (30mcg), Vancomycin-VA(30mcg), Amoxyclav-AMC(30mcg),Cotrimoxazole-COT(25mcg), Teicoplanin- TEI (30mcg) and linezolid-LZ(15mcg) **(Table 1 & 2)**.

All 8 different strains were found to be multidrug resistant organisms **(Figure 1)**. Their ESBL producing property is detected by double disk diffusion method **(Figure 2)**. Whereas **(Figure 3)** showing E test for Methicillin Resistance *Staphylococcus aureus.*

Susceptibility of Disinfectants

Out 25 samples, ten isolates were multi drug resistant in nature. This MDR strains showed different susceptibility pattern for the disinfectants used.

Gram positive organism (MRSA & VRE) was susceptible to Chloroxylenol, Benzylkonium chloride and sodium hypochlorite in the highest dilution of 1:320, whereas Chlorhexidine gluconate was effective only in the lowest dilution of 1:20.

Similar to the Gram positive organisms, Chloroxylenol, Benzylkonium chloride had same effects on Gram negative MDR organisms at an average dilution of 1:160 & 1:320 respectively. But for Chlorhexidine gluconate and Sodium hypochlorite showed inhibition pattern in the lowest dilution of 1:20 and 1:40.

Followed by those disinfectants, phenol was ineffective and exhibited resistant even in the first dilution1:20 for both gram positive and gram negative organisms.

In our study the most effective disinfectants was Chloroxylenol and Benzylkonium which inhibited the growth in the dilution of 1:320 **(Table 3)**. The two MDR organisms (*Pseudomonas* and *Acinetobacter sps*) that had been reported in various outbreaks of nosocomial infection in hospitalized patients **[10]** was found to be resistant to 3 out of 5 disinfectants which we have tested **(Figure 4 & 5)**.

Time Kill Assay

This MDR isolates were also tested by Time-kill assay technique from the viewpoint of

exposure duration. This technique was performed to evaluate the bactericidal effects of the 5 disinfectants. Chloroxylenol and Benzylkonium chloride showed 100% sensitive pattern with 1minute of contact time. Followed by Chlorhexidine gluconate and Sodium hypochlorite were also showed 100% inhibition at 10 minutes of contact time but in our study 'Phenol' was proved to be ineffective disinfectant for *Pseudomonas* and *Acinetobacter* species Whereas other isolates had 100% inhibition at 30 minutes of time.**(Figure 6)**.

Table 1: Antibiotic susceptibility pattern of Gram negative organisms

Clinical isolates	Antibiotic susceptibility pattern							
	GEN	AK	CTX	CZ	CIP	CAZ	IPM	AMC
E.coli	S	S	R	R	R	R	S	R
Klebsiella pneumonia	S	S	MS	R	R	R	S	R
Klebsiella oxytoca	R	S	R	R	R	R	S	R
Proteus mirabilus	R	R	R	R	R	R	S	R
Proteus vulgaris	R	R	R	R	R	R	S	R
Enterobacter aerogenes	S	S	R	R	R	R	S	R
Pseudomonas aeruginosa	S	S	R	R	R	R	S	R
Acinetobacterbaumanii	R	MS	R	R	R	R	S	R

R-resistant, S- sensitive, MS- Moderately sensitive

TABLE: 2 Antibiotic susceptibility pattern of Gram positive organisms

Clinical isolates	Antibiotic susceptibility pattern								
	OX	AMP	AK	CIP	CTX	CPM	VA	LZ	CN
Staphylococcus aureus	R	R	MS	MS	R	R	S	S	R
	AMP	AK	ERY	HLG	TE	CIP	VA	LZ	TEI
Enterococcus faecium	S	R	R	R	R	R	R	S	S

R-resistant, S- sensitive, MS- Moderately sensitive

Table 3: MIC of disinfectants against clinical isolates

Clinical isolates	MIC of the Disinfectants				
	Chloroxylenol	Benzylkonium chloride	Chlorhexidinegluconate	Phenol	Sodium hyphochlorite
MRSA	1:320	1:320	1:20	Growth till 1:20	1:320
VRE	1:320	1:320	1:20	Growth till 1:20	1:320
E. coli	1:240	1:320	1:20	Growth till 1:20	1:40
Klebsiella pneumonia	1:160	1:160	1:20	Growth till 1:20	1:40
Klebsiellaoxytoca	1:160	1:160	1:20	Growth till 1:20	1:40
Proteus mirabilus	1:160	1:320	1:20	Growth till 1:20	1:40
Proteus vulgaris	1:160	1:320	1:20	Growth till 1:20	1:40
Enterobacteraerogenes	1:160	1:320	1:20	Growth till 1:20	1:20
Pseudomonas aeruginosa	1:320	1:320	Growth till 1:20	Growth till 1:20	1:20
Acinetobacterbaumanii	1:320	1:320	1:20	Growth till 1:20	1:20

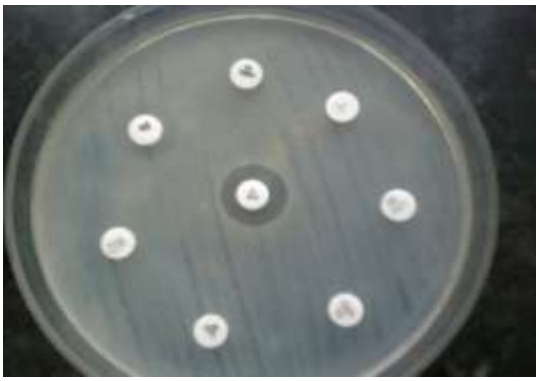

Figure 1: Kirby Bauer's Disk Diffusion Technique, Showing Multi Drug Resistant

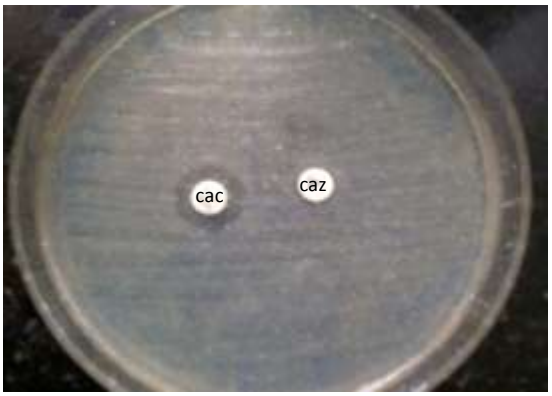

Figure 2: Double disc diffusion method, showing ESBL positive for Gram negative organism

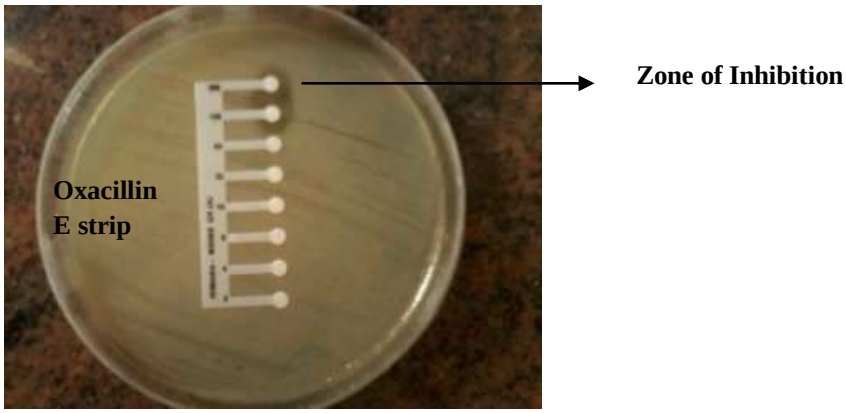

Figure 3: E- Test showing Oxacillin Resistance *Staphylococcus aureus*

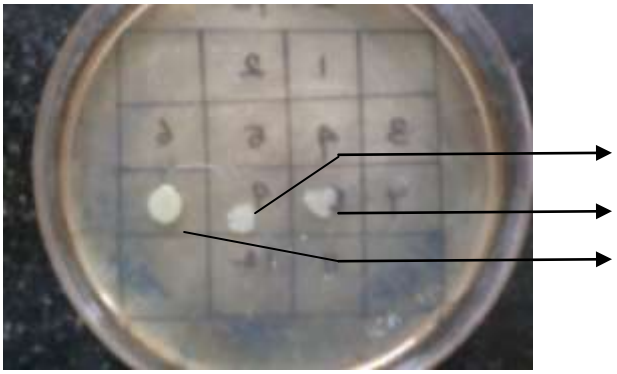

Figure 4: MIC of sodium hypochlorite with concentration of 40

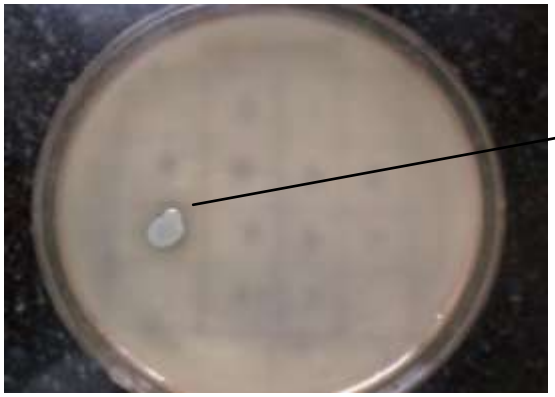

Figure 5: MIC of Chlorhexidine gluconate with concentration of 20

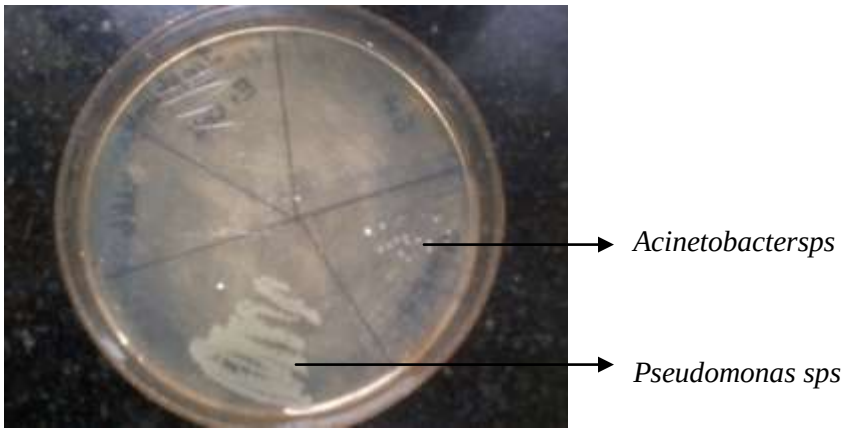

Figure: 6 showing the growth of *Pseudomonas* and *Acinetobacter sps* after 30 minutes of contact time with phenol

DISCUSSION

Hospital environment with infected patients are usually contaminated with potential MDR pathogens such as MRSA, VRE, ESBL, MBL, AmpC strains **[11-15]**. They survive in the in-animate object or hospital environment and

cause cross transmission between the patients with in the health care facilities. In recent times, there are various reports on increased emergence of resistance to antimicrobials and even disinfectants **[16]**. Nowadays it is difficult to treat infections caused by MDR strains.

In our study, the two methods: MIC and time kill assay were done to find out the effectiveness of commonly used disinfectants against MRSA,VRE, ESBL producing gram negative organisms and MDR *Pseudomonas* and *Acinetobacter* species.

Most important factors affecting efficacy of chemical disinfectants are the time of exposure and concentration used and type of bacterial isolate. Use of disinfectants with the contact time specified in the label is too long to be followed practically in a health care setting; commonest practice is to apply a disinfectant and allowing it to dry within one minute. Also there is always a chance of drying of the disinfectant on the surface even before exerting its disinfectant action at greater contact time. Hospital workers tend to follow the manufacturer's instruction in choosing concentration of disinfectants without knowing its efficacy.

To overcome, it is essential to perform efficacy testing of disinfectants regularly in health care settings. Routine practice of hand-hygiene and use of appropriate disinfectant with adequate contact time in treating hospital environment during any outbreak may possibly reduce the transfer of drug resistant organisms between the patients and healthcare workers.

In 2008 Nogbulie NJ *et al* compared the efficacies of various disinfectants like Lysol, Dettol, Purit, Roberts and Wexcide in 1:100 dilutions against *Streptococcus* species, *Staphylococcus* species, *E. coli*, *Pseudomonas* species and Bacillus species. They reported that the *Pseudomonas* species was susceptible to Lysol. *Streptococcus* species, *E.coli* and *Proteus* species was 80% susceptible to all the disinfectants used. Bacillus species was only 20% susceptible to all the disinfectants **[17]**. Similarly in our study it was proved that MDR *Pseudomonas* ,*E.coli*, *Proteus* was susceptible to Bezylkonium chloride.

Gopinath *et al*., (2013), evaluated the efficacy of disinfectants against Acinetobacter species, and also reported Benzylkonium chloride, chloroxylenol and sodium hypochlorite had good activity. Among which sodium hypochlorite found to have 100% susceptibility and inhibits the growth of *Acinetobacter* species within 10 minutes of contact time. This study reveals that there was no effect for phenyl compound against *Acinetobacter* **[18]**. Likewise our

investigation correlates with previous studies and proving the effectiveness of these three disinfectants and ineffectiveness for phenol against *Acinetobacter* and *Pseudomonas* species.

Among the 5 different disinfectants tested for bactericidal efficacy Benzylkonium chloride, sodium hypochlorite and Chloroxylenol showed better activity against both Gram positive as well as Gram negative organisms. Among all disinfectants, Phenol was ineffective disinfectant. This suggested that choice of appropriate disinfectants may play a vital role in controlling nosocomial infection.

CONCLUSION

The main aim of this project was to study the effectiveness of disinfectants against MDR strains. In most of the hospitals, cleaning is regularly conducted by less skilled workers. In addition, there are no rules or regulations for proper disinfectants. Disinfectants are often misused and rationalization of their use in hospitals is desirable for control of infection and costs. Therefore, in health care settings with high prevalence of MRSA, VRE and β-Lactam resistance strains may possibly use these disinfectants in spite of drug resistance to avoid cross contamination in the hospital environment!!!.

ACKNOWLEDGMENT

My sincere thanks to Ms. Anitha, for her immense support and encouragement during this study.

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