



Original Research Article

Efficiency of some commonly used disinfectants on bacterial isolates from critical areas of tertiary care hospital

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ABSTRACT

Aim of the study: Monitoring bacterial susceptibility to antimicrobials and disinfectants may help the management of nosocomial infection. Environmental surfaces are epidemiologically important reservoir of nosocomial bacterial species. The successful eradication of these pathogens with antibiotics has been complicated by the development of highly resistant strains as well as the appearance of new virulent pathogens.

Materials and Methods: Mop water with added disinfectant was collected from critical areas of the hospital which were- surgical operation theatre, ophthalmology operation theatre, and intensive care unit of medicine. Isolates were tested by surface test method for efficacy of 6 commonly used disinfectants; Sodium hypochlorite, Phenyl black, Povidone iodine, Lysol (cresol) - benzalkonium chloride, Chloroxylenol & Chlorhexidine for recommended dilutions.

Results: Majority of isolates were from M-ICU. Strains representative of GNB (gram negative bacilli) were most prevalent as compared to NFGNR. Recommended dilution of all the disinfectant was found satisfactory except phenyl black. Recommended dilution for phenyl black is 1:200 in water and as per our results of the study; MIC and MBC of organisms were found to be high. So it is recommended to use it in the critical areas of hospital with caution.

Conclusion: The problem of hospital infection is a real one. Antibiotic resistant NFGNR, especially *Pseudomonas* spp. have played a predominant role. Antimicrobial activity of disinfectants may be influenced by their formation, level of organic load, synergy, temperature, dilution rate and tests methods. Disinfectant testing strategies should be part of any healthcare infection control policy.

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1. Introduction

Ever since the identification of microorganisms as the causative agents of infectious disease, various methods have been developed to reduce the population and prevalence of these organisms. These various methods are like chemotherapy, immunization, sterilization and disinfection. The European Committee for Standardization of disinfectants has defined disinfection as the selective elimination of certain undesirable organisms in order to prevent their transmission achieved by action on their structure or metabolism, irrespective of their functional

state.¹ The pathogenic bacteria significantly contribute to human illness and death especially as a result of hospital acquired infections. The successful eradication of these pathogens with antibiotics has been complicated by the development of highly resistant strains as well as the appearance of new virulent pathogens.^{1,2} Environmental surfaces are epidemiologically important reservoir of nosocomial bacterial species.

Many chemical agents are now available commercially as disinfectants and antiseptics. These preparations include halogen compound, phenols and halogenated phenolic compounds, tar acid phenol, biguanids, alcohols, aldehydes, peroxygens and quaternary ammonium compound and chlorohexidine gluconate.^{2,3} The activity of disinfecting

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agents is affected by many factors like concentration of disinfectant, time of action, pH, temperature, formation procedure, phenol content, organic matter and quality of water used during dilution.¹

Hospital infections are widespread and have high economic and social impact. Bacteria associated with nosocomial infections are gram negative rods from *Enterobacteriaceae* family, non fermenting gram negative rods, *Staphylococcus*, especially *coagulase negative species* and *Enterococcus*. Monitoring bacterial susceptibility to antimicrobials and disinfectants may help the management of nosocomial infection. Microorganisms may be associated to several biological materials in the hospital environment such as floors, walls, ceiling, doors, windows, electro-electronic equipment and specific hospital articles in use for assistance to patients. Thus, the quality of cleaning services is an important condition in the prevention and control of microbial spread.⁴

The present study was carried out to test efficiency of some commonly used disinfectants on bacterial isolates from critical areas of tertiary care hospital.

2. Materials and Methods

2.1. Sample collection and processing

Mop water with added disinfectant was collected from three critical areas of the hospital- surgical operation theatre, ophthalmology operation theatre, and intensive care unit of medicine after institutional ethical clearance during the time period of Jan 2012 to March 2012.

2.2. Culture

The collected material was cultured on different culture media. For the colony count CFU/ml (colony forming unit per ml of sample), 0.1ml of sample was plated on TSB agar plates and colony count was done as per the given formula: CFU/ml = number of colonies × dilution factor/ volume plated. Mac Conkey's agar (Himedia lab., Mumbai, India) and blood agar were used for the isolation and presumptive identification of microorganism through morphological and biochemical characteristics according to the standard CLSI guidelines (2016). The isolated strains were maintained at -20⁰ C by inoculating colonies into vials containing tryptic soy broth with 30% glycerol. Antibiotic susceptibility testing was performed for all isolates by Kirby beur disc diffusion method.

2.3. Determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of the disinfectants against the isolates

The six hospital based disinfectants with their recommended dilutions checked by surface test method for the evaluation

of the bactericidal activity and the active ingredients were as follows- 1. Sodium hypochlorite (1:1000), 2. Phenyl black- phenyl (free from QAC and mercury) (1:200), 3. Povidone iodine- 5%w/v iodine (undiluted), 4. Lysol (cresol) - benzalkonium chloride (1:100), 5. Chloroxylenol- chloroxylenol and ethanol (1-10%), 6. Chlorhexidine- 4%w/v chlorhexidine gluconate, propanol, and ethanol (undiluted). The disinfectants were stored in dark at room temperature and various dilutions were prepared in sterile distilled water on the day of testing of MIC and MBC.

2.4. Inoculum preparation

Colonies of test organisms were transferred to 0.9ml of tryptic soy broth (TSB) (Himedia) and 0.1ml of bovine serum albumin (Himedia), till turbidity similar to Mac farland 0.5.

2.5. Surface test method

0.1ml of BSA and 0.9ml of test suspension N was prepared and 0.1ml of this mixture was put on the sterile glass slide and allowed to dry called it as carrier slide. Different dilutions of each disinfectant were prepared and dried slide of carrier was placed into the different dilutions of disinfectants for 2min. After that carrier slide was put into the 25ml of neutralizing solution for 5min called it as 'Na'. After 5min, 0.5ml of 'Na' was added into 4.5 ml of tryptic soy broth and incubated for 24hrs at 37⁰ C for determination of MIC. After 24hrs loopful of TSB suspension was cultured on TSB agar plates and incubated for 24hrs at 37⁰ C for determination of MBC. For water control 'Nw' same as 2nd step except hard water was used instead of disinfectant. To prepare validation suspension (Nv), 2ml of N suspension was added into the 6ml of TSB. 0.5ml of 'Nv' solution and 4.5ml of TSB was mixed to prepare 'Nv₀' solution. 0.5ml of this solution was spread on TSB agar plates and incubated. After incubation colony count was made.

3. Results

In the present study samples of mop water with disinfectants used for regular mopping in three critical areas of hospital (Medicine ICU, Surgery OT, and Ophthalmology OT) were collected. Total of 23 bacterial isolates out of 11 samples were recovered from the rinsed water of cleaning mops used at the critical areas of the hospital. Percentage of isolates obtained from Medicine ICU was 43.5%, Surgery OT was 37.1% and Ophthalmology OT was 17.1%. The percentage of various organisms isolated is shown in Chart 1. Among the isolates, 9(39.13%) were NFGNR, while 14(60.8%) were GNR. Distribution of various microorganisms isolated from different critical areas of the hospital is shown in Chart 1.

MIC and MBC of sodium hypochlorite for all the isolates was >1:1000, povidone iodine <1%, chlorhexidine >1:32,

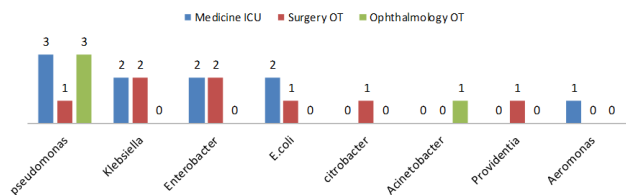


Chart 1: Distribution of isolated bacteria from different critical areas

Lysol >1:200 and chloroxylenol <1%. For phenyl black 1 isolate of *Pseudomonas spp.* had MBC of 1:100, 1 isolate of *Klebsiella spp.*, 2 of *Enterobacter spp.* and 1 of *E. coli* had MIC of MBC of 1:200, which were less as compared to recommendation.

Out of 10 antibiotics tested, *Pseudomonas spp.* was sensitive to 5 antibiotics like-cefepime, amikacin, gentamicin, ciprofloxacin, and tigecycline, while GNR were sensitive to cefepime, amikacin, tigecycline, trimethoprim. Most effective antibiotics were cefepime, amikacin, gentamicin, tigecycline for which susceptibility were-47.8%, 78.2%, 34.7%, 65.2% respectively. High level of resistance was shown by isolates against ampicillin, piperacillin, ceftriaxone, trimethoprim.

4. Discussion

Majority of isolates were from M-ICU (43.5%), and this may add to higher incidence of acquiring nosocomial infections amongst patients of M-ICU. In the present study representative of NFGNR (non-fermenting gram negative rods) were 39.13% while 60.8% were GNR which contradicts to the result of study Bouzada *et al*⁴, where CONS was the most prevalent (43.7%) isolated bacteria followed by representatives of the GNR (25.2%) and NFGNR (22.9%).

Sodium hypochlorite was the compound which showed the highest inhibition efficiency, one with recommended dilution of 1:1000 (0.1%). Bouzada *et al*⁴ has showed similar results and found all the isolates equally susceptible to 0.125% concentration of sodium hypochlorite. Awodele *et al*⁵ study showed inhibitory activities against all the tested organisms at 100% concentration. Attention should be given to the susceptibility of all strains to 1% sodium hypochlorite as it is widely used in hospitals.

MIC and MBC to phenyl black were found higher than recommended, so it should be used in the critical areas of hospital with caution. It is advisable to verify its recommended dilutions. Satisfactory results were obtained in case of povidone-iodine. The present study results showed that all the tested bacteria were susceptible to highly diluted (1%) povidone-iodine which indicates its effectiveness even after dilution, which contradicts to the result of Alsaimary *et al*⁶ stating that 50% concentration is

not effective to all genera of bacteria.

In case of chlorhexidine, though the recommended use is the direct application of it the present study showed effectiveness up to dilution of 1:32. The result also supports the findings of Olowe OA *et al*,³ where >1:30 dilution of chlorhexidine was efficient in eradicating many of bacterial strains. The result obtained proves Lysol to be effective in overcoming infection at a dilution concentration recommended by the manufacturer. Thus it is widely used in hospital settings. The isolates those showed resistance towards it, were only when it was diluted to double the time then the recommended concentration. As per the study conducted by Rutala WA *et al*,⁷ 1:10 dilution of Lysol was able to reduce the bacterial count to a great extent. It was also proved efficient for all gram negative as well as positive organisms, which supports the present study observation.

All the tested organisms in present study showed complete susceptibility towards chloroxylenol with the dilution concentration of <1%, like results of study done by Olowe OA *et al*³ in which 1% concentration of chloroxylenol was efficient in eradicating many of bacterial strains. Its efficacy indicates that it could prove to be beneficial when used in concentrations recommended by the manufacturers.

5. Conclusion

Medicine ICU is the most important area of hospital to be screened on regular basis for reservoirs of resistant microorganisms. Gram negative rods (GNR) and non-fermenting rods (NFGNR) with multidrug resistance are major nosocomial pathogens. Results obtained from present study confirm that concentration affects the activity of disinfectants. The different active components could have attributed to the difference in activity of the disinfectants. It has been recommended that disinfectants for general use should be able to kill a wide range of common or potential pathogens. On the other hand, the emergence of *Klebsiella spp.* showing resistance to the disinfectants is of interest as it has not been previously shown to survive in and contaminate disinfectants. Only fresh preparations of disinfectants should be used routinely and dilution should be restricted to the concentration ranges that have been found to have definite activity against the organisms. Phenyl black should be substituted or replaced by other more effective disinfectants in critical areas of hospitals. It should be our routine practice to check MIC and MBC of disinfectants at intervals to see their efficacy and its use in prevention and control of hospital acquired infection.

6. Source of Funding

None.

7. Conflict of Interest

None.

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