Investigation of the Efficacy of Some Disinfectants Against Nosocomial Gram Negative Bacteria

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Abstract

Nosocomial infections are an important cause of morbidity and mortality all over the world. Most bacteria that cause nosocomial infections can retain their viability even after exposure to disinfectants in routine practices. Therefore, evaluation of the efficacy of disinfectants at each hospital is important. The objective of the present study was to determine the susceptibilities of nosocomial Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa isolates to various disinfectants. Nosocomial isolates were obtained from Central Microbiology Laboratory, Ibn-i Sina Hospital, School of Medicine, Ankara University. Susceptibility testing was performed by the quantitative suspension test at contact times of 3, 5 and 10 minutes. According to the results, all of the isolates were found susceptible to 2% glutaraldehyde (2% GA), 4% chlorhexidine gluconate (4% CHG), 7.5% povidone iodine (7.5% PI), 10% povidone iodine (10% PI) and 70% 2-propanol (70% 2-P) at 3 minutes contact time. However, 4 x E. coli, 19 x K. pneumoniae and 30 x P. aeruginosa isolates were found susceptible to 3% hydrogen peroxide (3% HP) at 3 minutes contact time, 7 x E. coli and 10 x K. pneumoniae isolates were found susceptible at 5 minutes contact time and 11 x E. coli isolates were found susceptible at 10 minutes contact time. 4 x E. coli isolates were found resistant to 3% HP. In conclusion, 2% GA, 4% CHG, 7.5% PI, 10% PI and 70% 2-P can be safely used against E. coli, K. pneumoniae and P. aeruginosa owing to their high effectiveness, however, 3% HP should not be preferred against E. coli due to the presence of resistant isolates in Ibn-i Sina Hospital, Ankara University.

Keywords: Disinfectants, Nosocomial Infections, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa

1. Introduction

Nosocomial infections (NI) are a major problem throughout the world, associated with increased morbidity, high mortality and significant health care costs (McDonald, 1998 and Capretti et al, 2008). These infections develop during hospitalization and are neither present nor incubating at the time of the patient’s admission (Benenson, 1995 and Block, 2001). Antiseptics and disinfectants are broad-spectrum biocidal compounds that inactivate microorganisms on living tissue and inanimate surfaces (Theraud et al, 2004). They are an essential part of infection control practices (McDonnell et al, 1999 and Shimizu et al, 2002). NI can be prevented by proper disinfection practices (Guimaraes et al, 2000). Considering the importance of the prevention of NI, the aim of this study was to determine the susceptibilities of nosocomial Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa isolates to various disinfectants.

2. Materials and Methods

2.1. Materials

2.1.1. Bacterial Strains

The following nosocomial isolates of bacteria were obtained from Central Microbiology Laboratory, Ibn-i Sina Hospital, Faculty of Medicine, Ankara University between
June 2007-June 2008: 26 x E. coli (26 x blood isolates), 29 x K. pneumoniae (24 x blood, 2 x abscess, 2 x urine, 1 x sputum isolate), 30 x P. aeruginosa (10 x blood, 10 x tracheal aspirate, 5 x urine, 2 x abscess, 3 x sputum isolates).

Identification of bacterial strains were performed by classical identification methods and API 20 E System (bioMerieux, France). E. coli ATCC 25922, K. pneumoniae RKK 574, P. aeruginosa ATCC 27853 were used as control strains.

2.1.2. Disinfectants

The agents tested were 2% (w/v) glutaraldehyde (2% GA), 4% (w/v) chlorhexidine gluconate (4% CHG), 7.5% (w/v) povidone iodine (7.5% PI), 10% (w/v) povidone iodine (10% PI), 70% (v/v) 2-propanol (70% 2-P), 3% (w/v) hydrogen peroxide (3% HP). Sterile distilled water was used as a diluent and disinfectant control. The disinfectants were stored in the dark at room temperature.

2.2. Methods

2.2.1. Neutralization / Recovery System

Neutralizer efficacy is important for accurate determination of the efficacy of an antiseptic or disinfectant (Tunçay, et al, 2003). Neutralizer (0.5 % Tween 80 in Tryptase Soy Broth (TSB) (Merck, Germany)) was previously tested to determine whether it was appropriate to inactivate each of the chemicals (Griffiths et al, 1997). Firstly, 100 µl of sterile distilled water was added to 900 µl of the disinfectant at the highest use concentration, mixed and left for 1 minute then 10 µl of this mixture was added to 990 µl of the neutralization / recovery medium. 10 µl of the undiluted test suspension of E. coli ATCC 25922 was added to this mixture (neat), vortex mixed for 20 seconds and serially diluted to 10^{-3} in Ringer's solution. 100 µl of the neat and subsequent dilutions were spread onto Tryptase Soy Agar (TSA) (Merck, Germany) in duplicate, using sterile spreaders. The plates were incubated at 37° C for 24 hours and colony-forming-units (cfu) were enumerated. The undiluted test suspension was used as the initial count. The test was repeated using water instead of the disinfectant as the control. The neutralizer was deemed suitable as there was no difference in colony size, growth rate or the number of cfu retrieved from tests and controls. This shows the neutralization / recovery system was effective and not inhibitory.

2.2.2. Assessment of Disinfectant Activity

Susceptibility testing was performed by the quantitative suspension test (Tunçay, et al, 2003). A single isolated colony of bacteria was removed from TSA plates and grown separately in 10 ml of TSB for 24 hour at 37 °C. After incubation, the tubes were centrifuged for 20 minutes at 2000 rpm with a rotor centrifuge. The cell pellets were washed with 10 ml of TSB. Then bacterial suspensions in TSB were adjusted to the McFarland 0.5 standard. In brief, 100 µl of bacterial suspension was added to 900 µl of the disinfectant solutions at room temperature for contact times of 3, 5 and 10 minutes. Then at the end of the each contact time 10 µl was removed to 990 µl of the neutralization system and serially diluted to 10^{-3} to 10^{-5} times. 100 µl of each dilution was placed onto TSA plates in duplicate by the spread-plate technique and incubated at 37 °C for 24 hours. Then surviving colonies were enumerated and expressed as cfu per milliliter. The reduction rate was calculated as the expression of the disinfectant efficacy, according to the following formula:

\[
\log_{10} \text{reduction} = \log_{10} \text{pre-disinfection count} - \log_{10} \text{disinfection count}
\]

Log10 reductions of 5 or more were taken as an indication of satisfactory microbicidal activity.

2.2.3. Statistical Analysis

Fisher’s Exact Test was performed to determine if there was statistically significant difference between time in terms of the susceptibility probability. Minitab 15.0 [Minitab Inc., USA] statistical package was used for statistical analysis (p≤0.05).

3. Results and Discussion

3.1. Results

The results of the suspension tests are presented as log10 reductions of test bacteria after 3, 5 and 10 minutes of contact. All of the isolates were susceptible to 2% GA, 4% CHG, 7.5% PI, 10% PI and 70% 2-P at 3 minutes contact time. However, 4 x E. coli, 19 x K. pneumoniae and 30 x P. aeruginosa isolates were found susceptible to 3% HP at 3 minutes contact time, 7 x E. coli and 10 x K. pneumoniae isolates were found susceptible at 5 minutes contact time and 11 x E. coli isolates were found susceptible at 10 minutes contact time (Table 1). 4 x E. coli isolates were found resistant to 3% HP.

If we compare the contact time for 3% HP, there was no statistically significant difference between 3 and 5 minutes (p=0.499), 3 and 10 minutes (p=0.064), 5 and 10 minutes (p=0.382) for E. coli. For K. pneumoniae, the difference observed between 3 and 5 minutes (p=0.035) was statistically significant.
Table 1. Susceptibilities of Gram Negative Nosocomial Isolates to 3% HP

<table>
<thead>
<tr>
<th>Nosocomial isolates</th>
<th>Contact Time</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>3 minutes</td>
</tr>
<tr>
<td>E. coli (n:26)</td>
<td>4</td>
</tr>
<tr>
<td>K. pneumoniae (n:29)</td>
<td>19</td>
</tr>
<tr>
<td>P. aeruginosa (n:30)</td>
<td>30</td>
</tr>
</tbody>
</table>

3.2. Discussion

Direct contact between healthcare workers and patients is believed to be the primary mechanism in the spread of many pathogens. Improper disinfection practices can increase the risk of transmission and horizontal spread of the pathogens that often result in NI (Reitzel et al, 2009). Antiseptics are an important component in avoiding healthcare-associated infections which are connected to invasive procedures, such as surgery or intravascular devices insertion (Steczko et al, 2010). The widespread and uncontrolled use of antiseptics and disinfectants has prompted some speculation on the development of microbial resistance, in particular whether antibiotic resistance is induced by antiseptics or disinfectants (McDonnell, 1999). Gram-negative bacteria are generally less susceptible to biocides than Gram positive species. Such resistance is likely to be intrinsic rather than plasmid-mediated, due to outer membrane that acts as a protective barrier (Russell, 1997). The quantitative suspension test has been used to evaluate the antimicrobial activity of antiseptics and disinfectants (Liguori et al, 2009). This test clarifies a linkage between time and concentration used in the procedure (Tunçay et al, 2003).

The use of alcohol-based hand antiseptics has become a standard worldwide to prevent the transmission of nosocomial pathogens by the hands of the healthcare workers (Kampf et al, 2008). In the United States and Europe, both ethanol and isopropanol are recognized active agents in medicinal products as recommended by the Centers for Disease Control and Prevention (CDC) (Council of Europe, 2007 and U.S. Pharmacopeial Convention, 2009). The U.S. Food and Drug Administration (FDA) assessed aqueous ethanol at 60% to 95% and isopropanol at 70% to 91.3% (vol/vol) as safe and effective for patient preoperative skin preparations. In Europe, n-propanol is also approved as an active ingredient in medicinal products for skin antisepsis (Reichel et al, 2009). In this study, no resistance to 70% 2-P was detected among nosocomial E. coli, K. pneumoniae, P. aeruginosa isolates. Reichel et al (2009) reported that n-propanol was the most effective alcohol in reducing the aerobic skin flora. To achieve the same reduction as n-propanol, ethanol or isopropanol must be applied at higher concentrations or for longer times or both.

Adequate skin antisepsis before invasive procedures is essential because of complications that could result from bacterial contamination in an immunologically compromised area. PI solutions solutions are often used to provide such antisepsis (Birnbach et al, 1998). Karadenizli et al (2003) found that 7.5% PI was the most effective agent on nosocomial P. aeruginosa, K. pneumoniae, E. coli isolates. Shimizu et al (2002) reported that 10% PI showed high activity against nosocomial K. pneumoniae and P. aeruginosa isolates. In this study we also found no resistance to 7.5% PI and 10% PI among nosocomial E. coli, K. pneumoniae, P. aeruginosa isolates.

Bacteria can survive in PI solutions. Multiple use PI bottles may become contaminated by bacteria. Previously opened iodophor containing solutions may lose their antimicrobial effectiveness because of a partitioning of the iodine between the micelle structure of the surface active agent and the water phase (Birnbach et al, 1998). Because of that reason, if PI solution is chosen for skin antisepsis, only single use containers should be used.

According to the evidence-based practice in infection control (Pratt et al, 2007) and CDC guidelines (O’Grady et al, 2002), 2% CHG is the recommended agent to be used prior to invasive procedures. In this study we found no resistance to 4% CHG among nosocomial E. coli, K. pneumoniae, P. aeruginosa isolates. Although 2% CHG is able to significantly reduce intravascular catheter-related infections (Reichel et al, 2009 and Steczko et al, 2010) the chlorhexidine-alcohol combination displays activity higher than that of aqueous chlorhexidine solution or alcohol solution in a preoperative skin preparation and in vitro tests (Reichel et al, 2009). The superior clinical efficacy of chlorhexidine-alcohol is probably related to its more rapid action, persistent activity despite exposure to bodily fluids, and residual effect (Darouiche et al, 2010). Some studies also indicate that skin preparation with alcoholic chlorhexidene is more efficacious than skin preparation with aqueous povidone iodine in preventing catheter-relating bloodstream infections and. surgical-site infections (Mimoz et al, 1999 and Darouiche et al, 2010). The residual effect of chlorhexidine gluconate appears to provide an advantage over other disinfectants (Capretti et al, 2008).

The selection of the appropriate antimicrobial agent is a very crucial step before application. Together with efficacy, immediate action and persistance, the problem of direct and indirect tissue injury should be taken under account. The most common injuries include ototoxicity.

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skin irritation, ophthalmic damage and anaphylactic reactions. Also, depending upon the application, the effectiveness in the presence of blood, necrotic tissue, or purulence may be an issue (Steczko et al, 2010).

2% GA, which is a dialdehyde employed as a low-temperature disinfectant and sterilizer, mainly for endoscopes and surgical instruments. It has some adverse effects: direct contact can cause dermatitis and aggravate eczema, while the fumes can cause rhinitis and conjunctivitis (Vizcaino-Alcaide et al, 2003 and Miner et al, 2010). In this study we found no resistance to 2% GA among nosocomial E. coli, K. pneumoniae, P. aeruginosa isolates.

HP (H₂O₂) is a clear and odorless antiseptic liquid that rapidly decomposes into water and oxygen when it combines with organic tissue or blood. During this breakdown, HP produces effervescence that mechanically cleans wounds and removes tissue debris via the oxygen that is released. HP modulates many physiologic and pathologic processes, with many of its effects occurring during wound healing (Wasserbauer et al, 2008). It is a relatively stable oxidant but may be converted by neutrophils and macrophages to more reactive species such as superoxide and hydroxyl radicals. These highly reactive oxygen species have been suspected to adversely affect wound healing by causing cell membrane and DNA damage. Thus, several studies have actually found that HP may be more cytotoxic than bactericidal (Wasserbauer et al, 2008 and Pottage et al, 2010). Alt et al (1999) demonstrated that local treatment with 3% HP significantly reduced bacterial growth on polymer biomaterials even for 1 month after treatment. Beneduce et al (2009) reported that 3% HP was effective in reducing the numbers of both aerobic and anaerobic bacteria present on the toothbrush heads. In this study, susceptibility to 3% HP was varied. For E. coli, 4 isolates were resistant to 3% HP, but no resistance was detected between K. pneumoniae and P. aeruginosa isolates. Disinfectants and antiseptics must have sufficient contact time with the surfaces to which they are applied in order to allow them to kill the germs (McDonnell, 1999). In this study, it was found that antimicrobial activity increased with the contact time.

4. Conclusion

Due to differences between hospital environments and susceptibility of the isolated bacteria, resistance to commonly used disinfectants may develop, each hospital should define its own disinfectant usage policy. In conclusion, 2% GA, 4% CHG, 7.5% PI, 10% PI and 70% 2-P can be safely used against E. coli, K. pneumoniae and P. aeruginosa owing to their high effectiveness, however, 3% HP should not be preferred against E. coli due to the presence of resistant isolates in Ibn-i Sina Hospital, Ankara University, Turkey.

References


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