DIVERSITY OF BACTERIAL CONTAMINATION ON IN-ANIMATE SURFACES OF A TERTIARY CARE HOSPITAL AND THEIR SENSITIVITY TO DISINFECTANTS

H. M. Khan, S. Rehman, S. Sana*, M. A. Ali* and A. A. Anjum*

Department of Pharmacy, Lahore College for Women University, Lahore
*Department of Microbiology, University of Veterinary and Animal Sciences, Lahore
Corresponding author’s email: humairaphd@hotmail.com

ABSTRACT: Healthcare-associated infections are major causes of morbidity and mortality among hospitalized patients. These infections are associated with frequent inanimate surface contamination in hospitals. In the present study antibacterial activity of three commercially available disinfectants including Benzalkonium chloride (BZK), Polyhexamethylene biguanide (PHMB) and Glutaraldehyde C11-15 Pareth 9 against Escherichia coli, Pseudomonas spp., Serratia spp. and Vibrio spp. isolated from inanimate surfaces of urology ward were evaluated. Efficacy of disinfectants was determined by minimum inhibitory concentration (MIC) and agar well diffusion. Mean zones of inhibition (ZOI) of BZK against the isolates ranged from 23.8 to 26mm followed by PHMB 23.3 to 27.4mm and Glutaraldehyde C11-C19 Pareth 9 had minimum efficacy against E. coli, Pseudomonas spp. and Serratia spp. while Vibrio spp. were least susceptible to BZK. The mean MIC value of PHMB against E. coli, Pseudomonas spp. Vibrio spp. and Serratia spp. was the lowest among the disinfectants.

Key words: Healthcare associated infections, Disinfectants, Inanimate surfaces, Minimum inhibitory concentration and Agar well diffusion.

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INTRODUCTION

Gram negative bacteria frequently contaminate air, equipment and surfaces in hospitals (Otter et al., 2011). Health care associated infections (HCAIs) are major causes of morbidity and mortality in hospitalized patients (Rutala et al., 2006). Patients are considered as the major source of infection transmission among the individuals. Contaminated inanimate surfaces of hospitals are touched frequently by health care workers (HCWs) and patients act as a source of contamination (Rutala and Weber, 2001). Gram negative bacilli can cause different infections including respiratory tract, urinary tract, blood stream, surgical sites, gastrointestinal and soft tissue infections (Ghotaslo and Behram, 2012; Allerberger et al., 2002). These microorganisms are controlled by physical or chemical agents like as antiseptics, disinfectants and detergents. Routinely, the disinfectants such as phenols, chlorhexidine, hypochlorite, alcohol, glutaraldehyde, formaldehyde, hydrogen peroxide, peracetic acid, cupric ascorbate, sodium hypochlorite, quaternary ammonium compounds (QACs), organic mercurials, peroxycarbons (hydrogen peroxide, peracetic acid, ozone) and silver salts are applied on inanimate surfaces (Mazzola et al., 2003; Block and Furman, 2002; Penna et al., 2001 and Sagripanit et al., 1997). These agents are essential part of infection control practices and assist in the prevention of nosocomial infections (Ghotaslo and Behram, 2012). The Present study was designed to estimate the comparative efficacy of disinfectants against Gram negative bacteria isolated from polluted inanimate surfaces of urology ward, Mayo Hospital, Lahore.

MATERIALS AND METHODS

Collection of samples: The study was carried out in urology ward, Mayo Hospital, Lahore, Punjab, Pakistan. Swab samples (n=50) from non-porous surfaces (n=10, each from door handles, over bed tables, side tables, bed railings and side chairs of subunit-II, Urology ward were taken immediately after disinfectant application and drying. The samples were transported immediately to the Bacteriology laboratory, Department of Microbiology, University of Veterinary and Animal Sciences, Lahore and stored at 2-8°C (French et al., 2004).

Isolation and identification: Gram negative bacteria were isolated and identified following Bergey’s Manual of Determinative Bacteriology (1994). Cotton swabs were streaked on MacConkey’s agar plates under aseptic conditions and incubated aerobically at 37°C for 24 hours. The isolates were identified based on colony characteristics and biochemical profile including oxidase, indole production, methyl red, VP, citrate utilization, glucose fermentation, lactose fermentation, Na
requirement, triple sugar iron, motility tests and growth on EMB agar.

**Antibacterial activity of disinfectants:** Three commercially available disinfectants including Benzalkoniumchloride (13.2%) [BZK], Polyhexamethylenebiguanide (20%) [PHMB] and Glutaral C11-C15 Pareth 9 were evaluated for antibacterial activity against the bacterial isolates by agar well diffusion method (Sharada et al., 2013). A uniform bacterial lawn was prepared using standardized inoculum (0.5 MacFarland). A volume of 30µl of each disinfectant was poured in well. Plates were incubated at 37°C for 24 hours. Zones of inhibition (mm) were observed and measured (Johnson, 1995).

**Minimum inhibitory concentration (MIC):** Minimum inhibitory concentration was determined using 96 wells microtiteration plate as described by Barros et al. (2007). Briefly, 100 µL medium was poured in each well and each disinfectant was serially diluted two fold as BZK (320 µL/mL, 160µL/mL, 80µL/mL, 40µL/mL, 20µL/mL, 10 µL/mL, 5 µL/mL, 2.5 µL/mL, 1.25µL/mL and 0.625µL/mL) and PHMB and Glutaral C11-C19 Pareth 9 (64 µL/mL, 32µL/mL, 16µL/mL, 8µL/mL, 4µL/mL, 2µL/mL, 1µL/mL, 0.5µL/mL, 0.25µL/mL, 0.125µL/mL). The standardized inoculum (100 µL of each culture) was inoculated in each well. Plates were incubated at 37°C for 24 hours. Absorbance was measured at 600nm using ELISA reader.

**Statistical analysis:** Data obtained were analyzed using SPSS 16.1 software for windows version. Association between the exposure indices was calculated for each area and the relative susceptibility of microorganisms to disinfectants was assessed using one way Analysis of Variance (ANOVA) at 0.05 by Duncan’s multiple range posthoc test.

**RESULTS**

A total of 59 bacterial isolates was purified from 50 swab samples of inanimate surfaces i.e. door handles, over bed tables, side tables, bed railings and side chairs). Out of these 18(30.5%) were Gram negative. The Percentage of Gram negative bacteria recovered was maximum (27.78%) in bed no. 21-25 followed by 22.22% (bed no. 16-20), 16.67% (bed no. 11-15) and 5.56% (bed. 1-10), respectively.

The purified isolates were identified as Pseudomonas spp. 7(11.86%) followed by E. coli 6(10.16%), Vibrio spp. 3(5.08%) and Serratia spp. 2(10.16), respectively (Table 1).

Benzalkoniumchloride and polyhexamethylenebiguanide showed efficacy against all isolates of Pseudomonas spp., E.coli, Vibrio spp. and Serratia spp. Glutaral C11-C15 Pareth 9 showed

**Susceptibility pattern of isolates:** Efficacy against 14.28% isolates of Pseudomonas spp. followed by 33.33% of Vibrio spp. and 0% of E.coli and Serratia spp.

Zone of inhibition showed by Benzalkoniumchloride for Vibrio spp. (26mm) was highest followed by Pseudomonas spp. (25.6mm), Serratia spp. (25.5mm) and E. coli (23.8mm). Polyhexamethylene biguanide (PHMB) exhibited the maximum antibacterial effect on Pseudomonas species followed by E. coli, Serratia spp. and Vibrio spp. with average ZOI of 27.4mm, 26.2mm, 27.5mm and 23.3mm, respectively. The ZOI of Glutaral C11-C15 Pareth 9 was found to be 13mm for Serratia spp. followed by 12mm for Vibrio spp. and E. coli and 11.4mm for Pseudomonas spp. (Figure 1).

**Minimum Inhibitory Concentration:** The mean MIC value of BZK for E. coli was (8.33 ± 2.58) followed by Pseudomonas spp., (9.64 ± 5.48), Vibrio spp., (10.83 ± 8.78) and Serratia spp. (50 ± 0.00) respectively. The mean MIC value of PHMB for E. coli was (6.33 ± 5.12) followed by Pseudomonas spp., (3.40 ± 2.22), Vibrio spp. (2.00 ± 1.73) and Serratia spp. (1.00 ± 0.00) respectively. The mean MIC value of Glutaral C11-C19 Pareth 9 for E. coli was (20.00 ± 9.79) followed by Pseudomonas spp., (19.40 ± 9.07), Vibrio spp. (8.00 ± 0.00) and Serratia spp. (40.00 ± 33.94). PHMB had lowest mean MIC value for Serratia spp., followed by Vibrio spp., Pseudomonas spp. and E. coli among all of the tested disinfectants. Mean MIC value of BZK and PHMB for Pseudomonas spp., E.coli, Vibrio spp. and Serratia spp. Differed nonsignificantly while the mean MIC value of Glutaral C11-C19 Pareth 9 differed significantly for Pseudomonas spp. and E. coli (Table- 2, Figure 2).

### Table 1: Morphological and Biochemical Characterization of Bacterial Isolates

<table>
<thead>
<tr>
<th>Morphological and biochemical Tests</th>
<th>E. coli</th>
<th>Pseudomonas spp</th>
<th>Vibrio spp</th>
<th>Serratia spp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>Rod</td>
<td>Rod</td>
<td>Curved Rod</td>
<td>Rod</td>
</tr>
<tr>
<td><strong>Gram Staining</strong></td>
<td>G -ve</td>
<td>G -ve</td>
<td></td>
<td>G -ve</td>
</tr>
<tr>
<td><strong>Oxidase</strong></td>
<td>-ve</td>
<td>+ve</td>
<td></td>
<td>-ve</td>
</tr>
<tr>
<td><strong>Indole</strong></td>
<td>+ve</td>
<td>NA</td>
<td></td>
<td>-ve</td>
</tr>
<tr>
<td><strong>Methyl Red Test</strong></td>
<td>+ve</td>
<td>NA</td>
<td></td>
<td>+ve</td>
</tr>
</tbody>
</table>
VP Test  -ve  NA  NA  -ve  
Citrate Utilization Test  -ve  NA  NA  NA  
Glucose fermentation Test  NA  -ve  +ve  NA  
Lactose fermentation  +ve  -ve  -ve  +ve  
Na Requirement Test  NA  NA  +ve  NA  
TSI Test  NA  NA  NA  -ve  
Motility  NA  NA  NA  +ve  
Growth on EMB agar  +ve  NA  NA  NA  

Table 2: Minimum inhibitory concentrations (MICs) of the disinfectants against Gram negative bacteria

<table>
<thead>
<tr>
<th>Sr. #</th>
<th>Disinfectant</th>
<th>Pseudomonas spp.</th>
<th>E. coli</th>
<th>Vibrio spp.</th>
<th>Serratia spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzalkoniumchloride</td>
<td>9.64 ± 5.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.33 ± 2.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.83 ± 8.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Polyhexamethylene biguanide</td>
<td>3.40 ± 2.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.33 ± 5.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>Glutaral C11-C15 Pareth 9</td>
<td>19.4 ± 9.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.00 ± 9.79&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40 ± 33.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means carrying different superscripts differ significantly and the means carrying same superscripts differ non- significantly (p<0.05).

Figure 1: Zones of inhibition of disinfectants against Gram negative bacteria

Figure 2: Microtitre plate showing result of MIC of disinfectants against Gram negative bacteria.
DISCUSSION

Fifty nine isolates were purified from 50 swab samples of inanimate surfaces. Out of these 41 (69.5%) were Gram positive and 18 (30.5%) were Gram negative bacteria. Similar findings have been reported by (Russotto et al., 2015; Hammuel et al., 2011; Kramer et al., 2006 and Lemmen et al., 2004). In the present study, the percentage prevalence of E. coli and Pseudomonas spp. was 10.16% and 11.86% respectively, were comparable to the findings of (Garcia-Cruz et al., 2012) the percentage prevalence of Gram negative bacteria on in-animate surfaces was 9.17% for E. coli and 32% for Pseudomonas spp.

According to present study, all of the Gram negative bacteria were susceptible to BZK and PHMB disinfectants. The presented results corresponded with the findings of Rutala and Weber, (1997) in which a number of disinfectants used were considered bactericidal when used in appropriate concentrations.

PHMB exhibited the greatest antibacterial effect on Pseudomonas spp. followed by E. coli, Serratia spp. and Vibrio spp. with average Zones of Inhibition (ZOIs) of 27.4mm, 26.2mm, 27.5mm and 23.3mm respectively. The results were similar to the findings of Lee et al., (2004) who observed that PHMB inhibited the growth of Gram negative bacteria on inoculated plates. The ZOIs of Benzalkoniumchloride for bacterial isolates was more than 22mm as did in the study of Sharada et al., (1995). Pseudomonas spp. was found to have the smallest ZOI of 11.4mm in this study comparable to that of Kovacs et al., (1998) who established that the ZOI of Glutaral C11-C15 Pareth 9 was 11.3mm against Pseudomonas aeruginosa.

In a bacteriological medium, at a concentration of 20µg/mL, PHMB was effective against Escherichia coli and ineffective against Pseudomonas aeruginosa (Rosenthal et al., 1982). Results of this study were similar as well as in contrast to the findings of present study where 2-16µL/mL and 2-8µL/mL of PHMB was inhibitory to E. coli and Pseudomonas spp. respectively.

According to Michael and Graham, (2006) all concentrations of PHMB tested (2.5–15µg/mL) were bactericidal for E. coli. The MIC value of BZK ranged from 25-100µg/mL for E. coli followed by 100-500µg/mL for Pseudomonas spp. and 75-150 µg/mL for Serratia spp. The results were comparable to the findings of present study where MIC value of E. coli which ranged from 2-16µL/mL for PHMB. The results were also in contrast to the findings of present study where the MIC value of BZK ranged from 8.33µL/mL for E. coli, 9.64 µL/mL for Pseudomonas spp. and 5µL/mL for Serratia spp.

According to present study, MIC value of glutaral C11-C15 Pareth 9 for E. coli was the highest among the tested disinfectants value of MIC which lied within the range of 8-32 µL/mL which was far less than that of the findings of Osman et al., (2012).

Conclusion: Among the tested disinfectants used in this study, Benzalkoniumchloride and Polyhexamethylene biguanide were found effective against Gram negative bacteria isolated from urology ward. So, these disinfectants may be used to minimize the risk of nosocomial urinary tract infections in tertiary care hospital settings in future.

REFERENCES


