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

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ABSTRACT: Healthcare-associated infections are major causes of morbidity and mortality among hospitalized patients. These infections are associated with frequent in-animate surface contamination in hospitals. In the present study antibacterial activity of three commercially available disinfectants including Benzalkonium chloride (BZK), Polyhexamethylene biguanide (PHMB) and Glutaral C11-C15 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 Glutaral C11-C19 Pareth 9 12 to 13mm. The MIC ranges were 2.5-20µL/mL for BZK; 1-16 µL/mL for PHMB and 8-64 µL/mL for Glutaral C11-C19 Pareth 9. It was concluded that Glutaral 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, peroxygens (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 Sagripantil *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 polutted 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, tripple 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 non-significantly 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

Morphological and biochemical Tests	*E. coli*	*Pseudomonas spp.*	*Vibrio spp.*	*Serratia spp.*
Morphology	Rod	Rod	Curved Rod	Rods
Gram Staining	G -ve	G –ve	G -ve	G -ve
Oxidase	-ve	+ve	+ve	-ve
Indole	+ve	NA	NA	-ve
Methyl Red Test	+ve	NA	NA	+ve

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	
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

Sr. #	Disinfectant	MIC values (Means ± S.D.)			
		Pseudomonas spp.	***E. coli***	***Vibrio spp.***	***Serratia spp.***
1	Benzalkoniumchloride	9.64 ± 5.48^{a}	8.33 ± 2.58^{a}	10.83 ± 8.78^{a}	5.00 ± 0.00^{a}
2	Polyhexamethylene biguanide	3.40 ± 2.22^{a}	6.33 ± 5.12^{a}	2.00 ± 1.73^{a}	1.00 ± 0.00^{a}
3	Glutaral C11-C15 Pareth 9	19.4 ± 9.07^{b}	20.00 ± 9.79^{b}	8.00 ± 0.00^{a}	40 ± 33.94^{a}

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

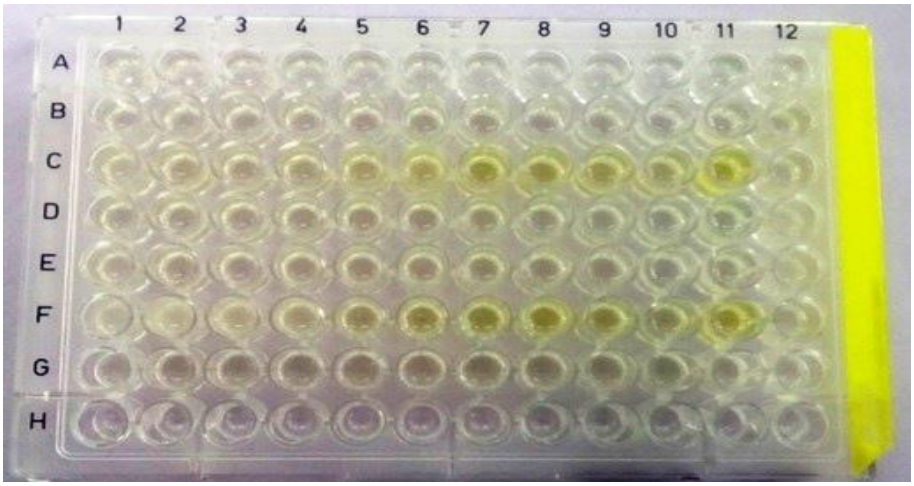

Figure 2: Microtitre plate showing result of MIC of disinfectants against Gram negative bacteria.

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

Allerberger, F., G. Ayliffe and M. Bassetti (2002). Routine surface disinfection in health care facilities: should we do it? Am. J. Infect. Control. 30(5): 318-327.

Barros, M. E. S., D. A. Daniel de Assis Santos and J. S. Hamdan (2007). Evaluation of susceptibility of *Trichophyton mentagrophytes* and *Trichophyton rubrum* clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A). J Med Microbiol. 56: 514–518.

Block, C. and M. Furman (2002). Association between intensity of chlorhexidine, use and micro-organisms of reduced susceptibility in a hospital environment. J. Hosp. Infect. 51(3): 201-206.

French, G. L., J. A. Otter, K. P. Shannon, N. M. T. Adams, D. Watling and M. J. Parks (2004). Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. J. Hosp. Infect. 57(1): 31–37.

Garcia-Cruz, C. P., M. J. N. Aguilar and O. E. Arroyo-Helguera (2012). Fungal and Bacterial contamination on Indoor Surfaces of a Hospital in Mexico. J. Mirobiol. 5 (3): 460-464.

Ghotaslo, R. and N. Behram (2012). Antimicrobial Activity of Chlorhexidine, Peracetic acid/ Peroxide hydrogen and Alcohol based compound on Isolated Bacteria in Madani Heart Hospital, Tabriz, Azerbaijan, Iran. Adv. Pharm. Bull. 2(1): 57-59.

Hammuel, C., E. D. Jatau and M. Z. Clement (2011). Prevalence and Antibiogram Pattern of some Nosocomial Pathogens Isolated from Hospital Environment in Zaria, Nigeria. Whong Aceh. Int. J. Sci. Technol. 3(3): 131-139.

Johnson, T. (1995). Chemical Methods of Control adapted from Laboratory Experiments in Microbiology (4th edition), Case C, Benjamin/Cummings Publishing Co. Redwood city.

Kovacs, B. J., R. M. Aprecio, J. D. Kettering and Y. K. Chen (1998). Efficacy of various disinfectants in killing a resistant strain of *Pseudomonas aeruginosa* by comparing zones of inhibition: implications for endoscopic equipment reprocessing. Am. J. Gastroenterol. 93(11): 2057-2059.

Kramer, A., I. Schwebke and G. Kampf (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect. Dis. 6: 130.

Lee, W. A., K. M. Tobias and D. A. Bemis (2004). *In vitro* efficacy of a polyhexamethylene biguanide-impregnated gauze dressing against bacteria found in veterinary patients. Vet. Surg. 33(4): 404–411.

Lemmen, S. W., H. Häfner, D. Zolldann, S. Stanzel and R. Lütticken (2004). Distribution of multi-resistant Gram-negative versus Gram-positive bacteria in the hospital inanimate environment. J. Hosp. Infect. 56(3): 191–197.

Mazzola, P. G., T. C. V. Penna and A. Martins (2003). Determination of decimal reduction time (D value) of chemical agents used in hospitals for disinfection purposes. Biomed Central Infect. Dis. 24(3): 147-157.

Michael, J. A. and F. Graham (2006). The response of *Escherichia coli* to exposure to the biocide polyhexamethylene biguanide. Morby. Microbiol. 152: 989–1000.

Osman, M., H. H. Hendawy, A. Hassan, S. M. Abdel-All and D. E. Mahmoud (2012). Efficacy of combination of glutaraldehyde and Benzalkoniumchloride against multidrug-resistant gram negative bacteria isolated from hospitals. J. Appl. Sci. Res. 8: 3019-3031.

Otter, J. .A., S. Yezli and G. L. French (2011). The Role Played by Contaminated Surfaces in the Transmission of Nosocomial Pathogens. Infect. Cont. Hosp. Ep. 32(7): 687-699.

Penna, T. C. V., P. G. Mazzola and A. M. S. Martins (2001). The efficacy of chemical agents in cleaning and disinfection programs. Biomed Central Infect. Dis. 1:16.

Rosenthal, I., B. J. Juven and E. Benhur (1982). Evaluation of poly (hexamethylene biguanide. HC1) as a biocide in the food industry. J. food safety. 4(4): 191-197.

Russotto,V., A. Cortegiani, S.M. Raineri and A. Giarratano (2015). Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. J. Intensive Care. 3: 54. doi:10.1186/s40560-015-0120-5.

Rutala, W. A. and D. J. Weber (1997). Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin. Microbiol. Rev. 10(4): 597-610.

Rutala, W. A. and D. J. Weber (2001). Surface disinfection: should we do it? J. Hosp. Infect. 48(9): 64-68.

Rutala, W. A., M. S. White, M. F. Gergen and D. J. Weber (2006). Bacterial Contamination of Keyboards: Efficacy and Functional Impact of Disinfectants. Infect. Cont. Hosp. Ep. 27: 372-377.

Sagripantil, J., C. A. Eklund, P. A. Trost, K. C. Jinneman, C. Abeyta and C. A. Kaysner (1997). Comparative sensitivity of 13 species of pathogenic bacteria to seven chemical germicides. Am. J. Infect. Control 25(4): 335-339.

Sharada, M., J. Ashok, H. Savitha, H. Mahesh and E. R. Nagaraj (2013). Comparative Effectiveness of Disinfectants with Phenol on Multidrug Resistant Bacteria and Fungi Isolated From the Clinical Sample - An In Vitro Preliminary Study. J. Evolution Med. Dental Sci. 2(18): 3055-3061.