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Research Article

Study the Efficacy of Antimicrobial Activities of Eight Clinically Applied Disinfectants against Clinical Isolated of Enterococci and *Pseudomonas aeruginosa*

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ABSTRACT

Background: Hospital-acquired infections are among the most significant reasons of human mortality world-wide which can be controlled by efficient application of suitable disinfectant for hospital setting. The main goal of the present study was to determine the efficacy of eight routinely used hospital disinfectants against clinical isolates.
Methods: In our descriptive study, in the first step the antibiogram assay of 99 clinical isolates enterococci and *Pseudomonas aeruginosa* were determined. Then, minimum inhibitory concentration and minimum bactericidal concentration of isolates against Povidone Iodine 10%, Ethanol 70%, Savlon 3.2%, Deconex51Gastro, Procept Floor, Septo med, Surfanious and Gigasept AF were evaluated. Furthermore, the efficacy of disinfectants was reevaluated in presence of 5% (w/v) Bovine Serum Albumin.
Results: The results showed that Septo med and Surfanious are the most and less potent disinfectants against clinical isolates, respectively. It is also resulted that Povidone Iodine is the most effective choice among the conventional disinfectants in this study. Clearly, addition of 5% organic substances reduced the efficacy of selected disinfectants significantly.
Conclusion: Novel quaternary ammonium compounds are the most applicable choice for disinfection of hospital surfaces and instruments in this study.

Introduction

Resistance to antimicrobial agents among bacteria is one the major world-wide challenge since the discovery of penicillin. Biocides are from antimicrobial agents which are divided to antiseptics, disinfectants and preservatives. The resistance level of various groups of bacteria to biocides were studied and characterized as well. Bacterial spores, mycobacteria and Gram-negative bacteria are insusceptible to biocides due to the presence of impermeable cell layers. Gram-positive bacteria, especially cocci are the most vulnerable types of microbes to biocides.[1,2]
Enterococcus spp. which are resistant to antimicrobial agents play an important role as nosocomial pathogens in hospital outbreaks.[3] As US nosocomial infections claimed, enterococcal disease ranked in among 3 or 4 most prevalent hospital-acquired disease. It is clear that enterococci are notable among hospitalized patients due to their pathogenicity by carrying virulence factors. Enterococcal urinary tract infections (UTIs), bacteremia and uncomplicated wound infections are the most common and meningitis as well as endocarditis is less frequent infections caused by these microorganisms.[4,5]
P. aeruginosa has emerged as an important cause of nosocomial infections among hospitalized patients, including pneumonia (hospital-acquired, healthcare-associated and ventilator-associated), burn infections, UTIs, meningitis and bacteremia. Unfortunately, developing antimicrobial resistance in *P. aeruginosa* due to acquisition of resistance genes on plasmids as well as intrinsic resistance

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make treatment of severe infections of *P. aeruginosa* quite problematic. Hence, selection of suitable antimicrobial agents is essential in health-care centers.[4,6,7]

In hospitals and health care facilities, biocides are widely used to prevent and control hospital-acquired infections. Disinfectants and antiseptics are applied to strongly decontaminate the microbial count of surfaces (solid surfaces and skin). However, there is a growing concern regard to maintaining the sanitary condition in hospitals. Because of the emergence of resistant bacteria to different antibiotics and silver compounds as well as widespread usage of disinfectants in hospitals, more researches are necessary to evaluate the efficacy of active biocidal substances not only on standard bacteria but also on clinical isolates.[4,8]

Furthermore, presence of organic materials such as mucous membranes and wounds is inevitable in health-care settings and as it is evidenced, the effectiveness of most of biocidal agents would reduce in contact to organic substances. Hence, the influence of such substances should be assessed in practical use of biocides.[9,10] There are many surveys on antibiotic resistance of bacteria while less research was focused on resistance to biocides. Therefore, the main goal of the present study was to determine and evaluate susceptibility of isolated enterococci and *P. aeruginosa* from hospitals to eight widely used disinfectants and biocides, which routinely used for disinfection of skins, surfaces, floors and facilities in the same medical centers both in presence and without organic substances.

Materials and Methods

Bacterial isolates

In our descriptive study, fifty isolates of enterococci and forty-nine isolates of *P. aeruginosa* were obtained from patients who were hospitalized at two state hospitals of Zanjan city of Iran from August 2012 to May 2013. Enterococci as well as *P. aeruginosa* were identified according to standard bacteriologic methods. All of enterococcal samples were recultured at our laboratory by incubated on bile esculin sodium azide agar for 18 h at 37 ºC.[11] Samples of *P. aeruginosa* were reidentified by cultured on cetrimide agar and P-agar.[12,13] In the next step, the single colonies saved in 30% glycerol at -80 ºC as stocks.

Antimicrobial susceptibility pattern of clinically isolated enterococci and P. aeruginosa

The susceptibility pattern of isolated enterococci and *P. aeruginosa* were evaluated by paper disc method.[14,15] Ten µl of (0.5 McFarland) enterococcal suspension was spread on surface of Mueller-Hinton agar plates. Then, the following discs of antibiotic purchased from Himedia, India were applied for enterococci: vancomycin (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), and streptomycin (10 µg). The antibiotics used for determination of susceptibility pattern of *P. aeruginosa* were tobramycin (10 µg), gentamicin (10 µg), ceftazidime (30 µg), imipenem (10 µg), ticarcillin (75 µg), levofloxacin (5 µg), kanamycin (30µg), piperacillin (100 µg) and aztreonam (30 µg). Subsequently, inhibition zones around disks were measured after incubation for 18 -24 hours. Quality control of tests was done by standard strains of *P. aeruginosa* PTCC 1310 and *E. faecalis* PTCC 1237.

Disinfectants and susceptibility testing

Eight different disinfectants which were used broadly in Zanjan state hospitals were obtained: Povidone Iodine 10% (PI), Ethanol 70% (Et), Savlon 3.2% (Sa), Deconex51Gastro (DG), Procept Floor (PF), Septo med (Sm), Surfanious(Sf) and Gigasept AF (Gi). The MICs of the above mentioned disinfectants were evaluated by broth micro-dilution method (microtiter assay) with reference to the protocol of the CLSI.[15]

Determination of bactericidal activity: MBCs

The MBCs were measured by plating and incubating 10 µl of four final clear diluted wells of each disinfectant at 37º C for 48 hours. MBC was defined as minimum concentration of disinfectants, which killed desired microorganisms.[10]

Measurement of bactericidal activities in the presence of organic material

The MBC test was repeated as described above in presence of 5% (w/v) Bovine Serum Albumin. It was applied to simulate presence of organic materials and evaluate bactericidal activity. The experiments were repeated three times on different days.[10]

Results

The antibiotic resistance pattern of 50 enterococcal strains isolated from patients was tested 82% of isolates were resistant to up to seven applied antibiotics. Regarding resistance pattern of antibiotics, seven different resistance patterns were shown in this survey. As shown in graph 1, 41 (82%) of enterococcal isolates were multi-drug resistant. Interestingly, the lowest prevalence of multi-drug resistance was 10% to three antibiotics. Resistance to five antibiotics (30%) was widely distributed among isolates, followed by 20% to four and 16% to two. As it is obvious from Figure 1 80% of *P. aeruginosa* were multi-drug resistant. Interestingly, 30% of isolates were resistant to 5 out of 9 different antibiotics. The most important result to get from the data is that 10% of isolates were resistant to almost all of the tested antibiotics.

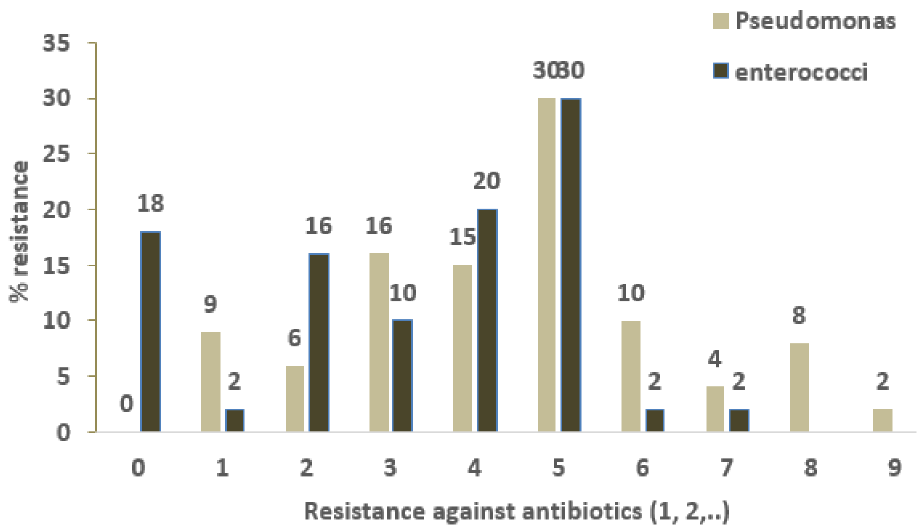

Figure 1. Antimicrobial resistance pattern of *Enterococcus* spp. and *Pseudomonas aeruginosa* isolated from clinical samples.

Table 1. Distribution of MIC (%), MBC (%) and MBCal (%) of various disinfectants by microtiter method.

Disinfectant	Number of strains at each MIC (%) of disinfectant											
	3.1×10^{-2}	1.5×10^{-2}	7.8×10^{-3}	3.9×10^{-3}	1.9×10^{-5}	9.7×10^{-4}	4.8×10^{-4}	2.4×10^{-4}	1.2×10^{-4}	6.1×10^{-5}	3×10^{-6}	1.5×10^{-5}
Sf -P	0	2	3	3	11	19	6	2	0	0	0	0
Sf - E	0	2	1	5	14	15	9	3	2	0	0	0
PF-P	1	0	1	1	0	10	16	14	0	0	0	0
Pf-E	0	0	0	0	2	8	21	11	6	2	0	0
Gi-P	0	1	1	3	6	18	13	4	0	0	0	0
Gi-E	0	0	0	0	8	13	17	9	2	1	0	0
DG-P	0	1	2	2	6	12	16	5	0	0	0	0
DG-E	0	0	0	2	7	9	17	9	4	2	0	0
Sm-P	0	0	0	1	2	1	2	3	14	14	6	1
Sm-E	0	0	0	0	0	1	3	4	20	13	6	3

Disinfectant	Number of strains at each MBC (%) of disinfectant											
	6.2×10^{-2}	3.1×10^{-2}	1.5×10^{-2}	7.8×10^{-3}	3.9×10^{-3}	1.9×10^{-3}	9.7×10^{-4}	4.8×10^{-4}	2.4×10^{-4}	1.2×10^{-4}	6.1×10^{-5}	3×10^{-6}
Sf -P	0	4	9	17	14	3	0	0	0	0	0	0
Sf - E	3	4	14	16	10	2	1	0	0	0	0	0
PF-P	0	2	2	10	17	11	4	1	0	0	0	0
Pf-E	0	0	2	8	17	13	7	3	0	0	0	0
Gi-P	0	2	5	16	17	6	1	0	0	0	0	0
Gi-E	0	0	5	12	18	12	2	0	0	0	0	0
DG-P	0	3	6	14	14	8	2	0	0	0	0	0
DG-E	0	2	7	9	16	9	6	1	0	0	0	0
Sm-P	0	0	0	2	3	6	10	21	5	0	0	0
Sm-E	0	0	0	1	2	4	17	17	5	4	0	0

Disinfectant	Number of strains at each MBC al (%) of disinfectant										
	1.2×10^{-1}	6.2×10^{-2}	3.1×10^{-2}	1.5×10^{-2}	7.8×10^{-3}	3.9×10^{-3}	1.9×10^{-3}	9.7×10^{-4}	4.8×10^{-4}	2.4×10^{-4}	1.2×10^{-4}
Sf -P	1	4	10	16	14	2	0	0	0	0	0
Sf -E	1	4	4	19	18	2	2	0	0	0	0
PF-P	0	2	4	10	15	12	4	0	0	0	0
Pf-E	0	0	3	7	13	18	7	2	0	0	0
Gi-P	0	2	6	21	13	5	0	0	0	0	0
Gi-E	0	1	5	8	20	13	3	0	0	0	0
DG-P	0	3	3	14	17	7	3	0	0	0	0
DG-E	0	2	6	8	15	12	6	1	0	0	0
Sm-P	0	0	2	5	7	11	16	5	1	0	0
Sm-E	0	0	1	2	4	15	19	6	4	0	0

Gastro (DG), Procept Floor (PF), Septo med (Sm), Surfanious (Sf) and Gigasept AF (SG), (P) Pseudomonas, (E) enterococci.

Table 2. Distribution of MBCs of various disinfectants by Agar-plate method.

Disinfectant	Number of strains at each MIC (%) of disinfectant						
	35	17.5	8.75	4.3			
Et-P	2	12	29	3			
Et-E	3	18	25	4			
	4×10^{-1}	2×10^{-1}	1×10^{-1}	5×10^{-2}	2.5×10^{-2}	1.2×10^{-2}	6.2×10^{-3}
Sa-P	2	5	11	21	6	2	0
Sa-E	0	2	3	16	16	10	3
	1.25	6.2×10^{-1}	3.1×10^{-1}	1.5×10^{-1}	7.8×10^{-2}	3.9×10^{-2}	
PI-P	2	6	22	15	2	0	
PI-E	0	4	17	19	8	2	

Disinfectant	Number of strains at each MBC (%) of disinfectant						
	70	35	17.5	8.75			
Et-p	15	23	8	1			
Et-E	12	32	5	1			
	8×10^{-1}	4×10^{-1}	2×10^{-1}	1×10^{-1}	5×10^{-2}	2.5×10^{-2}	1.2×10^{-2}
Sa-p	1	3	14	20	7	2	0
Sa-E	0	1	3	16	22	6	2
	2.5	1.25	6.2×10^{-1}	3.1×10^{-1}	1.5×10^{-1}	7.8×10^{-2}	
PI -p	2	5	28	10	2	0	
PI-E	0	2	18	21	7	2	

Disinfectant	Number of strains at each MBC al (%) of disinfectant						
	70	35	17.5	8.75			
Et-P	43	4	0	0			
Et-E	44	6	0	0			
	8×10^{-1}	4×10^{-1}	2×10^{-1}	1×10^{-1}	5×10^{-2}	2.5×10^{-2}	1.2×10^{-2}
Sa-p	2	12	25	7	1	0	0
Sa-E	1	2	16	24	6	1	0
	5	2.5	1.25	6.2×10^{-1}	3.1×10^{-1}	1.5×10^{-1}	7.8×10^{-2}
PI -p	1	7	22	14	3	0	0
PI-E	0	1	17	21	9	2	0

Povidone Iodine 10% (PI), Ethanol 70% (Et), Savlon 3.2% (Sa), (P) Pseudomonas, (E) enterococci.

It was understood from Table1 and 2 that the distribution of MICs of DG, PF, Sm, Sf, SG, PI, Et and Sa for 100 clinical isolates of enterococci and *P. aeruginosa*. From the data in Table 1, it is apparent that the hospital disinfectants are significantly more killing on isolated enterococci than *P. earoginosa*. As Table 1 shows, Sm is the most potent disinfectant which strongly inhibits the growth of clinical isolates. Furthermore, the growth of approximately 78% of isolates was inhibited at 9.7×10^{-4} % by the rest of disinfectants except Sm. However, Sf is the less potent ones among hospital disinfectant. It can be understood from Table 2 that PI is the most effective choice among the conventional disinfectants in this study.

The bactericidal activity of eight selected disinfectants was evaluated by agar plate assay after addition of neutralizer to the microtiter plate. The data of MBCs from Table 1 and 2 can be compared with data of MICs in Table 1 and 2, which shows that bactericidal concentration is approximately eight times less than inhibitory concentrations. Interestingly, Sm revealed high potency for bactericidal activity against clinical isolates.

The comparison of bactericidal activity of the eight disinfectants for enterococci and *P. aeruginosa* under the so-called conditions (clean and dirty) showed that the MBC values were increased up to half of the in-use concentrations in most of the disinfectants in presence of 5% BSA (Table 1 and 2). However, there was no significant difference between MBC of DG, GI and PF in dirty and clean conditions for isolated enterococci.

Discussion

Nosocomial infections caused by enterococci and *P. aeruginosa* are the main problems in hospitals since 1980 and as a result of this issue medical expenses are a burden for health care systems.[16] The studies show that efficacy of antibiotics and disinfectants are gradually reduced.[17,18] Inappropriate usage, inaccurate concentration and lack of sufficient training for preparation and storage of hospital disinfectants are among the important reasons of prevalence of resistance of disinfectants.[18-20] In comparison to many antimicrobial resistance researches about antibiotics, there is not so many global researches regard to resistance to biocides. Because of the clinical importance of enterococci and *P. aeruginosa* the efficacy of eight hospital disinfectants were evaluated against clinical isolates of enterococci and *P. aeruginosa*.

The results showed that Gram-positive bacteria in this study (enterococci) were more susceptible to disinfectants in comparison to Gram-negative bacteria (*P. aeruginosa)*. The outer membrane of Gram-negative bacteria inhibits or seriously reduces the penetration of molecules of disinfectants into cells.[21] Interestingly, *pseudomonas* genus contains structural factors, enzymes and toxins which are the main reasons of resistance to antibiotics and disinfectants.[22]

Septomed showed the strongest bactericidal activity against isolated enterococci and *P. aeruginosa* followed by DG, PF and Gi.

Overall, ethanol exhibited the highest MBC against isolates. High MBC in this study corroborates earlier findings by Mansouri (2006), Mitiku (2014), Alkolaibea (2015) which suggested ethanol as weakest disinfectant in comparison to cetrimide-C and Betadin.[23-25]

As it is obvious, the best activity was observed from new generation of disinfectants. This finding is in agreement with Amini's (2012), Saharkhizan's (2014) and Azma's (2015) findings, which found QACs such as Deconex, Descocid and Decocept as strongest antibacterial agents in clinic, therefore it is highly recommended that QACs should be applied for disinfection of instruments and critical surfaces in hospitals.[26-28]

These compounds were manufactured in 1990s and today they considered as compounds which are strongly effective against types of microorganisms. Interestingly, they have several unique characteristics such as broad-spectrum activity, without smell, colorless, heat resistant, with low toxicity and considered as very good detergents.

It is interesting to note that as previous studies showed the rate of resistance in Zanjan hospitals is notably low in comparison to hospitals of other cities of Iran such as Uromiyeh, Tabriz, Tehran or other countries such as Cuba and Pakistan.[29-33] Our investigations hypothesized that there are some main reasons regard to this occurrence. Firstly, majority of disinfectants which are prepared and used in hospitals of Zanjan are strong and fast acting ones. Secondly, the staffs changed the type of disinfectants used for different purposes (facilities, instruments, floors, etc.) every 6-9 months. Thirdly, maintaining of appropriate and sufficient education for precise preparation and practical application of disinfectants to the staffs.

Conclusion

In conclusion, the results of MIC and MBC of applied disinfectants in two state hospitals of Zanjan against clinical isolates of enterococci and *P. aeruginosa* showed that Septomed (Sm) is the best antimicrobial agents applied in two state hospitals of Zanjan and conventional antimicrobial agents such as Povidone Iodine 10%, Ethanol 70% and Savlon 3.2% are amongst the less effective ones.

It is recommended that further research be undertaken to evaluate the applicability of these disinfectants against other important clinical microorganisms such as *Escherichia coli* and *Staphylococcus aureus.* Furthermore, it would be interesting to assess required contact time for each disinfectant.

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Conflict of interests

The authors claim that there is no conflict of interest.

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