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ORIGINAL ARTICLE

The Effectiveness of Commonly used Antiseptics against Bacteria Isolated from environmental samples of the three Educational Hospitals in Hamedan city

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ABSTRACT
Nosocomical infections (NI) is considered as a major problem in healthcare around the world, as it increases hospital stay and health care cost. Incorrect using of disinfectants and antiseptics in hospitals is considered to be an important factor in development of NI. In the present study, the prevalence of different bacteria in 384 samples collected from three education hospitals of Hamedan city was identified, and based on the mood of the effectiveness (poor, intermediate and strong), the antibacterial activity of some commonly used antiseptics were investigated. Antiseptics were including; Sidex, Sodium Hypochlorit, Kereolin 2.5%, Hygiene 1%, Betadine, Savlon 3.2%, Chlorohexedine 1%, Deconex, Halmid and Microton. Our results showed that from total samples, 253 cases (65.88 %) were bacterial positive cultures, which was indicative of a high rate of bacterial infection in investigated hospitals. Staphylococcus epidermidis, Escherichia coli, Klebsiell were three most prevalent bacteria. A table containing the information on the most effective antiseptics against each bacteria and the percentage of influenced population from the overall bacterial population was provided. In conclusion, our findings provides a suitable basis for selecting the usual antiseptics in routine disinfection procedures and suggest that permanent identification of bacterial population is a useful activity in reduction of nosocomia;l infections.
Key words: nosocomial infection, antiseptic effectiveness, bacterial infection, disinfection.

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INTRODUCTION

Complications in results of nosocomical infections (NI) is considered as a major problem in healthcare around the world, as it increases hospital stay and healthcare cost [1, 2].
Microorganisms exist in different health care units, may spread in poor hygienic conditions and lead to development of infection in patients and health care staff. Due to the progress in development of disincentive and antiseptic techniques, prevalence of NI in most developed and developing countries had been decreased in recent years. However, according to the statistics, NI remains still as a major cause of mortality worldwide [3, 4].
Hospitalization, particularly when is associated with intensive care unit (ICU) admission and invasive procedures are two factor that increase the risk of infection [5].
Historically, staphylococci, pseudomonads, and Escherichia coli have been the three common NI; nosocomial pneumonia, wound infections following surgery and vascular access–related bacteremia are the leading causes of the most mobidity and mortality in hospitalized patients [6].
Previous study conducted on 14996 patients in hospitals of Germany (1997) revealed that the overall prevalence rate of nosocomical infections was %3.5, which occurred as follow; urinary tract infection (42·1%), lower respiratory tract infection (20·6%), surgical site infections (15·8%) and primary sepsis (8·3%). The highest prevalence rate (15·3%) was found in intensive care ward patients, followed by surgery (3·8%), general medicine (3·0%) and gynaecology/obstetrics (1·4%) [7].
In an another nationwide survey conducted in 1996 in France, Of the 236 334 patients in 830 participating hospitals, 6.7% presented with at least one nosocomial infection and 1.3% with an NI imported from another hospital. Nosocomial infection prevalence was particularly high in rehabilitation and long-term care facilities, especially for urinary tract and skin/soft tissue infections. Postoperative

patients accounted for 18% of the overall population and had twice the frequency of NI as other patients. The prevalence of methicillin-resistant Staphylococcus aureus was 0.6% and accounted for 57% of all S. aureus isolated from NI [8].

Improper use of antiseptics and sterilization methods for decontamination of patient care items and medical equipments is one of the important reasons behind development of infections in different units of hospital such as intensive care unit (ICU) [9] and critical care unit (CCU) [10] and emphasizes the necessity for effective application of disinfectants and sterilizers [11]. Therefore, a line of research has been about to find more effective ways of taking advantages of disinfecting and sterilizating methods or finding new antiseptics and antibacterial in order to limit NI.

Adams et.al reported that application of 2% (w/v) chlorhexidine gluconate (CHG) in 70% (v/v) isopropyl alcohol (IPA) for skin disinfection results in enhanced skin antisepsis when compared with three commonly used CHG preparations [0.5% (w/v) aqueous CHG, 2% (w/v) aqueous CHG and 0.5% (w/v) CHG in 70% (v/v) IPA]. [12].

Results from previous studies indicate that the risk of catheter-related bloodstream infection is reduced by one-half using chlorhexidine gluconate solution for vascular catheter insertion site in comparison to povidone iodine [13, 14].

Another study indicates that the introduction of alcohol/chlorhexidine hand hygiene solution (ACHRS) combined with education and motivation programs can improve hand hygiene compliance and reduce total nosocomial infections [15]. Furthermore, Johnson et. al reported that a 36 month period hand hygiene culture-change program using ACHRS intervention in five clinical areas of a large university teaching hospital that had high levels of methicillin-resistant *Staphylococcus aureus* (MRSA) leads to a reduction in the burden of nosocomial MRSA infections [16].

In alignment with these investigations, in the present study we made an attempt to evaluate the degree of effectiveness of some commonly used antiseptics and disinfectants against bacterial colonies isolated form ICU, CCU, burn ward and operation rooms of three educational hospital in Hamedan city .

MATERIALS AND METHODS

Study outline

Based on the statistical calculations, 384 samples were collected from the environmental surfaces of three educational hospitals (mainly; Mobashe Kashani, Fatemiyeh and Ekbatan), in Hamedan, Iran. NI samples were cultured in suitable media and after determining the existing strains, their sensitivity to usual antiseptics and sterilizers were surveyed, using disk diffusion method.

Sampling and bacterial infections

Sampling carried out randomly, 3 times a week, during a period of 3 months. Sampling was conducted by rubbing a sterilized swap on different points in ICU, CCU, burn ward and operation rooms, and then dipping the swap in 1ml of sterile solution of physiological salt solution as a carrier medium. The solution then was kept for future experiments. Bacterial infection in this study was recognized based on a standard criterion in which, environmental infection is defined as a condition that more than 10 microorganisms exist in an area of 1 cm^2. As sampling from a surface with an area of 1 cm^2, is practically difficult, we decided to do it in four different points ($5\,cm \times 5\,cm$) with an overall area of 100 cm^2, instead. Dividing by 100, we then offset the figure.

Identification of bacterial stains

Nutrient broth prepared in previous step was transferred to a Petri dish and cultured in blood agar medium. The number of microorganisms was assessed based on the number of colonies formed in the medium. Each colony was representative of one bacterium in the broth medium. When divided by 100, it reveals the number of microorganisms per 1 cm^2 of area from the surface it was sampled. Differrentional culturings and biochemical tests were applied for identifying the strains of bacteria.

Determination of antibacterial activity by the disk diffusion method

According to the protocol recommended by the National Committee for Clinical Laboratory Standards (NCCL) [17], the antibacterial activity of a number of commonly used antiseptics and sterilizing agents against the prevalent isolated strains of bacteria in the present study was separately determined using the disk diffusion agar medium. Briefly, a colony of each bacterial strain was diluted with a suspension of sterilized Mueller-Hinton (MH) broth to a final concentration of approximately 10^5-10^6 UFC/ml. Petri dishes containing MH agar medium were seeded with 5 min culture of the bacterial strains in nutrient broth. Antiseptics and sterilizing agents in a concentration of their usual use were applied separately to sterile filter paper disks (6 mm in diameter) and after incubation in 37 ◦C for 5 min, disks placed on the surface of the medium with a distance of 15cm between two of them. The plates were incubated at 37 ◦C for 24 h. A disk free of any antiseptics and sterilizing agent was placed on the middle of the medium, and considered as the negative control. The effectiveness of antiseptics or sterilizing agent- stained disks were

measured based on the diameter of the area around the disk that no bacterial colonies survived and it was categorized as follow;
<7mm; no effect, 7-10 mm; poor effect, 11-15 mm; intermediate effect and 15 < Strong effect. Antiseptics used in this study was as follow; Sidex, Sodium Hypochlorit, Kereolin 2.5%, Hygiene 1%, Betadine, Savlon 3.2%, Chlorohexedine 1%, Deconex, Halmid and Microton.

RESULTS AND DISCUSSION

Our findings showed that from 384 samples collected 253 cases (65.88%) were positive to bacterial infection. The prevalence of bacteria was shown in table 1, the bacterial infection regarding their prevalence was as follow order; Staphylococcus epidermidis, Escherichia coli, Klebsiella, Diphtheroid, Streptococcus group D, Acinetobacter, Bacillus subtilis, Enterobacter, Staphylococcus aureus, Micrococcus, Pseudomonas aeruginosa, Citrobacter, Pneumococci, Proteus, alpha hemolytic Streptococcus and Lactobacillus.

Table 1. Prevalence of bacteria collected from three education hospitals in Hamedan.

Bacterium name	Mobasher. K	Ekbatan	Fatemieh	Total	%
Staphylococcus epidermidis	19	25	25	69	17
Escherichia coli	11	11	11	33	8
Klebsiella	11	11	11	33	8
Diphtheroid	9	9	15	33	7
Streptococcus group D	11	8	8	27	6.8
Acinetobacter	5	11	11	27	6.8
Bacillus subtilis	10	8	8	26	6.5
Entrobacter	6	9	9	24	6
Staphylococcus aureus	11	7	7	25	6
Micrococcus	11	6	6	23	5.7
Pseudomonas aeruginosa	15	3	3	21	5.2
Citrobacter	6	7	7	20	5
Pneumococci	3	4	4	11	2.7
Proteus	3	3	3	9	2.2
alpha hemolytic Streptococcus	2	2	2	6	1.5
Lactobacillus	1	1	1	3	0.75
Total	141	128	128	397	100

The information about the total effectiveness of antiseptics and the mood of effect (poor, intermediate or strong) on the bacteria observed in collected samples of three educational hospitals in Hamedan city, and also the proportion of bacterial population affected by those antiseptics were gathered, and data for every bacterium were filled in a separated questionnaire (data not shown).
Table 2 is an essence of our findings about the most effective antiseptics (categorized on the mood of effect) against the prevalent bacteria investigated in the present study and was derived from the questionnaires.

Table2. The mood of effectiveness of antiseptics on prevalent bacteria collected from three educational hospitals in Hamedan.

Bacterium	Poor effect	Medium effect	Strong effect
Staphylococcus *epidermidis*	Savlon 3.2% (15.5%)*	Sodium Hypochlorit (6.9%)	Sodium Hypochlorit (3.4%)
Escherichia coli	Hygiene 1%, Betadine, Halamid (10%)	Microton. (16.7%)	Sidex (10%)
Klebsiella	Deconex (7.4%)	Deconex (14.8%)	Sidex (11.1%)
Diphtheroid	Halamid (11.1%)	Halamid (11.1%)	Sidex, Microton (14.8%)
Streptococcus group D	Halmid, savlon 3.2 %, Betadine	Deconex (11.5%)	Microton (5%)
Acinetobacter	Kereolin (7.7%) Hygiene 1% (8%)	Savlon %3.2 (12%)	Kereolin, Microton, Sidex (4%)
Bacillus subtilis	Betadine (16.6%)	Kereolin, Sodium Hypochlorit (8.3%)	Kereolin, Sodium Hypochlorit (4.1%)
Entrobacter	Savlon 3.2 % (13%)	Deconex , Betadine (9.4%)	Microton (9.4%)
Staphylococcus aureus	Savlon3.2 % (15.6%)	Hygiene 1%. Microton (8.7%)	Sodium Hypochlorit, Betadine (8.7%)
Micrococcus	Hygiene 1%, Halamid (11.5%)	Betadine, Chlorohexedine 1% (7.7%)	Sidex, Sodium Hypochlorit, Kereolin 2.5%, (7.7%)
Pseudomonas aeruginosa	Betadine, Halamid, Deconex (%7.5)	Sodium Hypochlorit (14.8%)	Sidex (3.7%)
Citrobacter	Halamid, Hygiene 1% (5%)	Hygiene 1% (20%)	Sidex (10%) Betadine, Microton (11.7%)
Pneumococci	Halamid (5.8%)	Halamid, Sidex (11.7%)	

*** The percentage of the bacteria is affected by the antiseptics.**

The present study was conducted in an attempt to identify the prevalent bacteria cause the nosocomial infections in the three educational hospitals and also the evaluation of effectiveness of commonly used antiseptics against them. Our results showed that Staphylococcus epidermidis was the most prevalent bacterium among the samples collected. In recent decades this bacterium has been considered as the common cause of a number of nosocomial infections [18]. Most of these infections occur in association with use of medical devices such asurinary tract, intrathecal and intravascular catheters and a spectrum of other polymer ans metal implants [19]. In the other hand , although Staphylococcus epidermidis is primarily a commensal bacterium it, however the increasing antibiotic resistance of its nosocomial isolates and also their of Staphylococcus aureus empowere the infectious ability of these bacteria which may be the reason behind their dominance in nosocomial infection cases [20, 21]. Although our findings do not propose Staphylococcus aureus as a most prevalent bacterium among the bacteria isolated in this study however, the medical history shows global pandemics of penicillin- resistance staphylococcus aureus-associated nosocomial infections occurred during 1950-60 decades [22]. . MRSA has become the most frequent cause of skin and soft tissue infections presenting to emergency departments in the United States [23]. Staphylococcus aureus is the cause of almost 20 % of bloodstream infections in the hospital setting [24] Data from table 1 shows that E. coli and Klebsiella are the second and third rank order

regarding the prevalence. In a previous prospective nationwide surveillance of nosocomial bloodstream infections in US hospitals, E. coli and Klebsiella were founded to be the cause of 6 % and 5% of infection, respectively [25].

Escherichia coli and *Klebsiella pneumoniae* are leading causes of serious infections in neonates, neutropenic cancer patients, and other patients with underlying diseases. Results of a previous study showed that extended- spectrum -lactamase (ESBL) producing strains of Klebsiella pneumonia and E. coli are responsible for outbreaks caused by these organisms in cancer centerrs, pediatric and geriatric wards, and hospitalized nursing home patients [26, 27].

Staphylococcus epidermidis was the most prevalent bacterium in the samples investigated in the present study and among antiseptics used in this study sodium hypocholorite, represents its inhibitory effect on growth of a proportionally more percentage of Staphylococcus epidermidis compared to other antiseptics. Results of a comparative analysis of the bactericidal activity of some disinfectants against antibiotic-susceptible and antibiotic-resistant hospital bacterial isolates in Brazilian hospitals showed that a 50% dilute of sodium hypochlorite solution was one of the antiseptic agents that was effective against both standard and also antibiotic-resistant hospital strains of Staphylococcus epidermidis and also other bacteria like Staphylococcus aureu, Enterococcus, Enterobacter cloacae, Proteus mirabilis and Escherichia coli [28].

Inanother study study bactericidal peptide 2 (BP2) which is a small synthetic antimicrobial peptide that its antibacterial potency in prevention and treatment of Staphylococcus epidermidis- biomaterial-associated infections (BAI) was observed [29].

According to Connell JF Jr et. al "The ideal antiseptic is one that is rapidly lethal to all forms of bacteria and their spores, capable of bactericidal activity for a prolonged period, has no injurious effects on wound tissues or skin, delineates the operation site and is easily applied and removed" [30] . in this regard, Kehinde O Elijah et. al , in their study propose that the use of iodine-based antiseptic agent like Betadinee in addition to Savlon guarantees better and more efficient disinfection than using Savlon alone to clean three times. As, the use of savlon twice followed by further disinfection with Betadinee reduced positive microbiological culture rate to 2.6% compared to 11.4%, in the occasion that Savlon used alone [31].

Kampf G et. al showed that hand disinfectants with a combination content of alcohol and chlorhexidine are more effective against methicillin-resistant *Staphylococcus aureus* (MRSA) than scrubs based only on chlorhexidine [32].

In conclusion, our findings evaluated the effectiveness of antiseptics usually is used in medical centers and indicates that none of antiseptics had enough potency to eradicate all the bacteria population in collected samples. Therefore, our study suggest that identifying the diversity of population in nosocomical infections and using a combination of antiseptics based on their effectiveness against different bacteria, would guarantee more effectiveness in routine disinfection procedures of medical environments.

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