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ARTICLE

INVESTIGATING THE EFFECT OF FREE ALDEHYDE/PHENOL ANTIBACTERIAL TO CONTROL THE COMMON HOSPITAL-ACQUIRED INFECTIONS INCLUDING PSEUDOMONAS AERUGINOSA, STAPHYLOCOCCUS AUREUS, ESCHERICHIA COLI, ACINETOBACTER, ENTEROCOCCUS

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ABSTRACT

Present wok is addressed for the evaluation of the efficiency rate of different free aldehyde/phenol antibacterial for the common hospital-acquired infections including Pseudomonas Aeruginosa, Staphylococcus aureus, Escherichia coli, Acinetobacter, and Enterococcus. Based on the results, the maximum and minimum phenol-coefficients were provided for Deconex 53 Plusand Saya sept HI, respectively. However, Deconex 53 Plus and Saya sept HI were the strongest and weakest disinfectants, respectively. Final disinfection under laboratory condition was performed, and it can be concluded that the fifth- and fourth- generation of quaternary ammonium compounds (Saya sept HI and Saya sept HP) showed better effects on hospital-acquired infections.

KEY WORDS
Hospital-acquired infections; free aldehyde/phenol disinfectants; MIC; MBC; Phenol coefficient

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INTRODUCTION

Many years ago, Socrates had noted to obstetric infections and prevalence, and in the past century because of undesirable health consequences of these infections to nursing homes and hospitals for diseases such as tuberculosis, smallpox, plague and etc. have become important. Today, because of health promotion these diseases have been approximately eradicated. But main concern about hospital-acquired infections is the opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Beside the direct control of these hospital-acquired infections, in a report of (Kyaw et al., 2015) it were found higher rehospitalization rates for patients with *Staphylococcus aureus* and *Pseudomonas aeruginosa* pneumonia. They were annually estimated that the hospital readmissions cost Medicare is about $17.5 billion. According to Centers for Disease Control and Prevention (CDC) database (in 2002), it has been estimated that >1.7 million hospital-acquired infections occur in hospital patients annually, that this is encompassed about 5% of all the admitted patients to a hospital (Calfee, 2012).The most common nosocomial infections includes: *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Staphylococcus aureus* and other non-fermentative Gram-negative bacilli (Hsueh et al., 2005; Obbard and Fang, ; Talebi et al., 2007). Based on the cost analysis estimation that have been reported by (Scott, 2009), it is revealed the overall direct medical costs of hospital-acquired infections in the U.S. hospitals ranging from $28-$45 billion each year. Expensive medical costs of the hospital-acquired infections including readmission, rehospitalization, drug and other related costs has caused the control and disinfectant application to be considered as a basic strategy. Although the use of disinfectants may increase the amount of bacterial resistance (Meyer and Cookson) and, it is costly, the application of proper dosage based on a regular plan can be one of the effective control methods. In this regard, to effective use of disinfectants and prevention of infection risk, Spaulding (1968) suggested three categories of germicidal action as follows: were noncritical, semi-critical and critical (Rutala and Weber, 2001).However, the application of suitable disinfectant regent and method should be considered regarding to control costs, efficiency and decrease of the bacteria resistant to disinfectant. The commonly used disinfectants for devices are including glutaraldehyde, ortho-phthalaldehyde, formaldehyde, chlorine and chlorine-releasing compounds, hydrogen peroxide, peracetic acid, alcohols, phenolics, quaternary ammonium compounds (QACs), and other germicides (Rutala and Weber, ; Simon et al., ; Widmer et al., 2006). Interface between infectious agents and infected person environmental surface such as devices and equipments. From the non-critical items or devices including blood pressurecuffs, bedpans, bed rails, crutches, linens, foodutensils, bedside tables, patient furniture and floors (Rutala and Weber, 1999). Low level disinfectants such as QACs, alcohols, Iodinated compounds and free-phenolic/aldehyde disinfectants arecommonly are used for these items. These compounds were upgraded to improve the disinfection characteristics. Fifth-generation of QACs is a sample of these promotions. These promotions were performed such as removal of the benzene ring, addition of substituent alcohols, amine group and etc. In this regard, the present work was conducted. The aim of present study was to evaluate the susceptibility of five hospital-acquired infections such as

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter*, and *Enterococcus* using the different free aldehyde/phenol disinfectants including new ones (such as *Saya sept HI, Deconex 50 AF*and *Septi turbo*) and conventional disinfectants (such as *Saya sept HP, Deconex 53 Plus* and *Deconex Solarsept*). To investigate the effect of each disinfectants minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and phenol coefficient (PC) were determined. The second aim the present work was to determine the efficiency performance of disinfectants under laboratory condition.

MATERIALS AND METHODS

Material

All regents and materials were prepared from authentic company in analytical grade. All disinfectants were provided from Behban Shimi Company, Iran, Borer Chemie AG, and Switzerland. As it can be seen in [Table 1], the characteristics of the used disinfectants are shown.

Bacterial culture detection

Firstly, the samples were cultured in Mueller Hinton Broth medium and were incubated in 37 ˚C for 24 h**,** then for identification of the each bacterium the specific medium was considered. For *Escherichia coli* the EMB medium was used. The green metallic luster after 37 °C for 24 h was indicated the presence of E. coli in sample. At similar conditions, Mannitol salt agar medium for detecting the *Staphylococcus aureus* was utilized. The Cetrimide agar, Bile Esculine Agar, OF medium were applied to detect and counting the *Pseudomonas aeruginosa, Enterococcus, Acinetobacter* spp., respectively.

Phenol coefficient (PC)

Phenol coefficient test was developed by Rideal-Walker 1903 and it is originally considered for determining the germicidal efficiency of disinfectants as compared to phenol, and it can be expressed as the following equation:

To determine the PC about 0.2 mL of *Salmonella typhi* was added to different dilutions (1:10, 1:20, 1:30, 1:40, 1:50 and 1:60) of phenol for 24 hours; then, a certain loopful of each dilution was transferred to sterilized tubes containing Mueller Hinton Broth medium at 5, 10 and 15 interval. After incubating the growth of infectious at 37 °C for 48 hours, it was monitored.

Determination of MIC and MBC

The minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) were determined according to the micro-dilution method [19]. The lowest concentration which can inhibit the growth of exposing bacteria to antimicrobial agent is considered as the minimum inhibitory concentration (MIC). MBC represents the lowest concentration of an antibacterial agent to kill 99.9% when compared to the MIC dilution.

Disinfection under laboratory condition

For evaluation of the disinfectant materials efficiency prior to application in fields this section was considered. To simulate a medicine center such as hospital units a surface such as the ceramic tiles for present work was selected. These tiles with dimension about 15×15 cm were prepared. In the first step, the tiles were rinsed and sterilized in autoclave. Then, to ensure from sterilization tiles surface was taken sampling onto ceramic tiles. All samples were cultured in blood agar medium and were incubated in 37 ˚C for 24 h. Next, 100 µL of the bacterial strains (0.5 McFarland) were added to tile surface and using the Sterile culture swabs bacterial strains was dispersed onto tile surface. Then, infectious tiles were disinfected by disinfectant materials with different dilutions as [Table 2]. Finally, sampling and cultivating after three times of disinfection about 15, 30 and 45 minutes in blood agar were performed. [Fig. 1] represents the photographic image of disinfection process and positive sample.

RESULTS

Antimicrobial test

To assess the killing potential, the phenol coefficient was determined for both of the disinfectants (new and conventional one). It can be seen in figure 2 that there is significant amounts in phenol coefficient for all type of disinfectants. The phenol coefficients were calculated about 1.33, 0.13, 0.53, 0.27, 1 and 5.8 for *Septi turbo*, *Saya sept HI*, *Deconex 50 AF*, *Saya septHP*, *Deconex Solarsept* and *Deconex 53 Plus*, respectively. The results showed that the relative germicidal efficiency of *Deconex 50 AF*, *Saya septHP* and

Saya sept HI are more than phenol under laboratory conditions. Also, the germicidal efficiency for *Deconex Solarsept* equals phenol and the other disinfectants were relatively weak to killing the *salmonella typhi* rather than phenol. The MIC of different disinfectants against hospital infectious strains is displayed in [Table 3]. Accordingly, to MIC values can be seen disinfectants concentrations and their dilutions for each infection. *Staphylococcus aureus* and *Escherichia coli* were more vulnerable than other infectious. To access minimum inhibition for *Enterococcus*s trains higher dosage of disinfectants were required. Regarding the minimum inhibition values, it is clear that the *Saya sept HI* and *Deconex Solarsept* are stronger to inhabit all infectious strains. Among them, *Saya sept HP* with lower dilutions and more amounts of disinfectant mater was recognized as the weakest. Originally, the phenol coefficient method is testing disinfectants against *Salmonella typhi*. This antimicrobial assay have been tested using *Salmonella choleraesuis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*(Oule et al., 2008). According to results [Fig. 2], it can be found that maximum and minimum phenol coefficient is related to *Deconex 53 Plus*and *Saya sept HI*. This result is demonstrated among tested disinfectants *Deconex 53 Plus*and*Saya sept HI* are the strongest and weakest, respectively. Both disinfectants are from ammonium quaternary compounds category, and this difference probably is related to disinfectant nature and their chemical formula.

Table 1: The characteristics of used disinfectants

Trade name	Characteristics	Trade name	Characteristics
Deconex 50 AF	Ingredients;-didecyldimethylammonium chloride, N,N-Bis(3-aminopropyl)-dodecylamine, coco propylenediamine-1.5-bis-guanidinium acetate -For disinfecting floors, walls and medical devices	*Deconex Solarsept*	Ingredients;- propan-2-ol, propan-1-ol, N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine -Ready-to-use , alcohol-based, rapid disinfectant -For wipe or spray disinfection of small areas and medical equipment
Saya sept HI	- International Code: 2424 ISIC, 353 CPC -Fourth-generate QACs - For disinfecting dental and medical tools - Ingredients; Dimethyl benzyl ammonium chloride, Alkyl dimethyl benzyl ammonium chloride	*Saya sept HP*	- International Code:2424 ISIC, 353 CPC -Fifth-generate QACs -Usage surfaces Effective on mycobacterium, virus and air-borne bacteria
Septi turbo	Ingredients; Deionized water, propanol, anticorrosion,Didecyldimethylammonium chloride, protective materials (70% alcohol and 25% QACs) -antibacterial, HIV, HBV -Fast-acting	*Deconex 53 Plus*	- Ingredients; alkyl propylene diamine guanidinium diacetate N,N-Didecyl-N-methyl-poly(oxyethyl)ammonium propionate -Aldehyde-free instrument disinfectant -For the pre-cleaning and disinfection of medical instruments, including rigid and flexible endoscopes

Table 2: disinfectants dilution for laboratory condition

Disinfectants	Dilution	Disinfectants	Dilution
Deconex 50 AF	1	*Deconex Solarsept*	1:4
	1:16		1:8
	1:64		1:16
Saya sept HI	1	*Saya sept HP*	1:8
	1:16		1:32
	1:32		1:64
Septi turbo	1:8	*Deconex 53 Plus*	1:4
	1:16		1:8
	1:32		1:16

Table 3: MIC results

Spp.	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Escherichia coli*		*Acinetobacter*		*Enterococcus*	
Disinfectant	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)
Septi turbo	1:16	12.5	1:64	3.1	1:64	3.1	1:16	12.5	1:16	12.5
Saya sept HI	1:128	1.5	1:256	0.75	1:128	1.5	1:128	1.5	1:64	3.1
Deconex 50 AF	1:32	6.2	1:64	3.1	1:64	3.1	1:32	6.2	1:8	25
Saya sept HP	1:16	12.5	1:32	6.2	1:32	6.2	1:16	12.5	1:8	25
Deconex Solarsept	1:128	1.5	1:265	0.75	1:256	0.75	1:128	1.5	1:128	1.5
Deconex 53 Plus	1:64	3.1	1:128	1.5	1:128	1.5	1:64	3.1	1:16	12.5

To determine the minimum bactericidal concentration (MBC), the MIC samples were cultured in Mueller-Hinton Agar medium and were incubated for 24 hours. After this time, no colony was growth. As a result, the MIC is considered equal to MBC [Table 4]. Based on minimum bactericidal and inhibitory concentration of disinfectant mater can be asserted: *Saya sept HP >Septi turbo> Deconex 50 AF> Deconex 53 Plus > Saya sept HI > Deconex Solarsept.* Also, *Saya sept HP* was recognized as the weakest disinfectant due to higher amount of disinfectant concentrations. *Staphylococcus aureus* and *Escherichia coli* were more vulnerable strains and *Enterococcus* were more resistance to minimum inhibitory concentration. This fact can be resulted from bacterial characteristics. For example, disinfectant resistance of *Enterococcus*s trains (such as *Enterococcus faecalis* and *Enterococcus faecium*) is provided by production of peptidoglycan cell wall precursors (Muto et al., 2003). Some strains of *Staphylococcus aureus* also can secrete extra cellular compounds (mucoid) and it can cause resistance to disinfectant agents. Generally, Staphylococci strains are considered as susceptible to disinfectant agents, but they are resistant to some disinfectants encompassing *ethidium bromide*, *acriflavine*, quaternary ammonium compounds (such as *cetrimide* and *benzalkonium chloride*), and *diamidines* (such as *propamidine isethionate* and *diamidinodiphenylamine dihydrochloride*)(Al-Masaudi et al., 1991) .Tennent and colleagues (1989) have reported about the genetic analysis of *Staphylococcus aureus* and its resistance characteristics (genes *qac*A-E) to antiseptics and disinfectants(Tennent et al., 1989).The quaternary ammonium compounds are low-level or weak disinfectants and they are not effective on spore forms. On the other hand, several mechanisms is facilitated the resistance of microorganisms to disinfectants agents that can be asserted as following: modifications in the membrane composition, expression of stress response and repair systems, or expression of efflux pump genes(Hegstad et al., 2010). Despite the low efficiency of quaternary ammonium compounds, there is widespread usage due to inoffensive characteristic (non-phenol and non-aldehyde).

Table 4: MBC results

Spp. / Disinfectant	*Pseudomonas aeruginosa*		*Staphylococcus aureus*		*Escherichia coli*		*Acinetobacter*		*Enterococcus*	
	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)	Dilution	Disinf. Dose (µg/mL)
Septi turbo	1:16	12.5	1:64	3.1	1:64	3.1	1:16	12.5	1:16	12.5
Saya sept HI	1:128	1.5	1:256	0.75	1:128	1.5	1:128	1.5	1:64	3.1
Deconex 50 AF	1:32	6.2	1:64	3.1	1:64	3.1	1:32	6.2	1:8	25
Saya sept HP	1:16	12.5	1:32	6.2	1:32	6.2	1:16	12.5	1:8	25
Deconex Solarsept	1:128	1.5	1:265	0.75	1:256	0.75	1:128	1.5	1:128	1.5
Deconex 53 Plus	1:64	3.1	1:128	1.5	1:128	1.5	1:64	3.1	1:16	12.5

Saya sept HI mechanism disinfectant is completed through adhesion and it disrupts the cytoplasmic membranes and the vital cell functions. In this compound, there is not benzene ring on didecyldimethylammonium chloride. As a result, it is more stable than other quaternary ammonium compounds and it can be caused high synergy and antimicrobial effects. Despite all the disinfectants, *Deconex Solarsept* is a 'ready-to-use' and alcohol-based disinfectant. Because the stabilizing of blood and dirty on surface when the alcohol-based disinfectant used, the water-based disinfectants are better to act in hospital and medicine center. Also, the use of water-based antimicrobial material is decreasing its effect by interferences such as water hardness, organic and inorganic agents. Based on MIC result, the *Saya sept HP* was introduced as the weakest disinfectants. This fact can be demonstrated by nature of *Saya sept HP*, and it could be caused by water hardness during MIC test analyzing. Since the cultured resulting plates of MIC were growing lower than 15 colonies, so the MBC can be equaled to MIC values [Table 4].

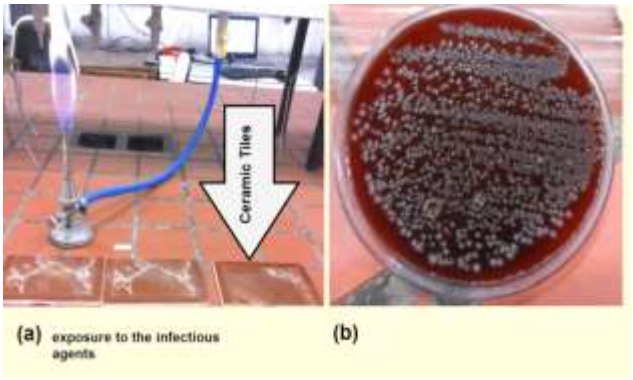

Fig. 1: Effect of disinfectants under lavatory condition (a), positive growth on blood agar (b).

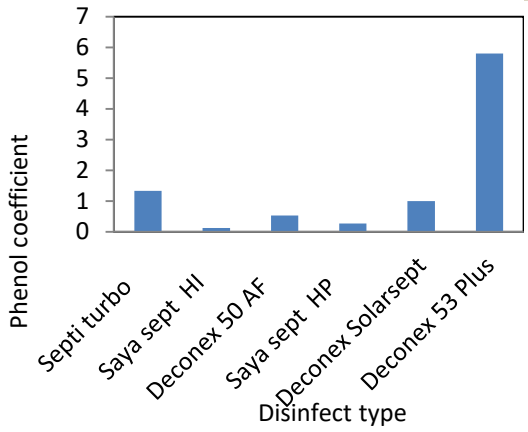

Fig. 2: Phenol coefficient for used disinfectants.

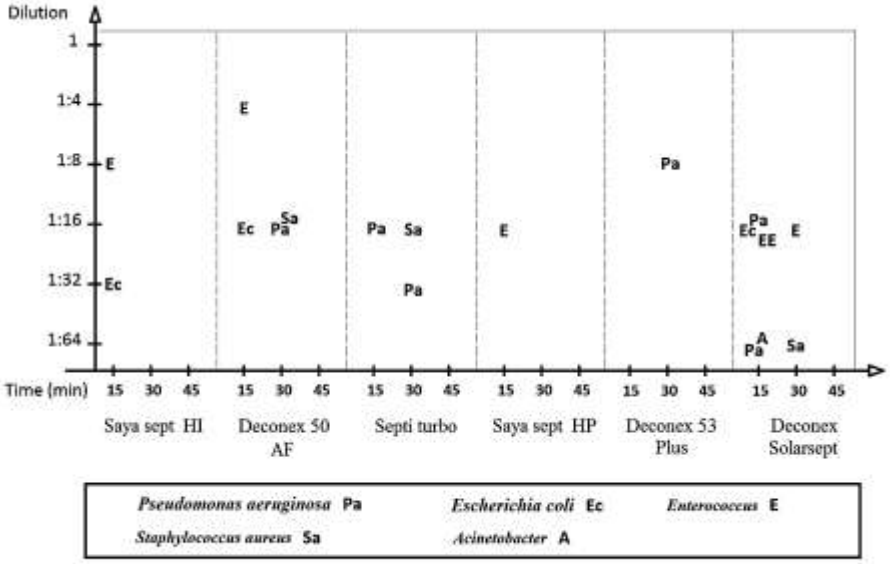

Fig. 3: Disinfectants killing potential evaluation under lavatory condition.

Disinfection under laboratory condition

In the next stage, under laboratory condition the killing efficiency of this mater were studied. At this time, the positive growth of infectious strains was survived at different concentration [Table 2] via three times of 15, 30 and 45 min. The result of this step is represented in [Fig. 3]. Regarding this, it can be seen higher killing potential of *Deconex 53 Plus*and*Septi turbo* for *Enterococcus*. In presence of *Saya sept HP* and *Deconex 53 Plus* grew only *Enterococcus* and *Pseudomonas aeruginosa*, respectively. Higher indicator strains were detected when *Deconex Solarsept*, *Deconex 50 AF*and*Septi turbo* was used. In most cases, at inadequate exposure time (<45) min the infectious strains growth can be seen. In all cases it is clear that *Saya sept HI*andthe *Saya sept HP* was able to control *Pseudomonas aeruginosa***.** In some dilutions, regeneration of indicators in presence of *Deconex 53 Plus*, *Deconex Solarsept*, *Deconex 50 AF* and *Septi turbo* was observed. At all dilutions in presences of *Deconex 53 Plus*, *Saya sept HI* and the *Saya sept HP* have controlled the *Staphylococcus aureus*. Also, *Escherichia coli* were controlled by *Deconex 53 Plus*, *Septi turbo* and *Saya sept HP* in all dilutions. According to results, regeneration phase was not observed for *Enterococcus*, *Acinetobacter* and *Escherichia coli* under laboratory condition. When the *Deconex Solarsept* was considered *Acinetobacter* growth was rather. On the other hand, *Enterococcus* was not seen when *Deconex 53 Plus* and *Septi turbo* were added. Similarity, Ghasemi and colleagues (2012) have reported that *Deconex* was an effective material when it was used for disinfection of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* (Ghasemi et al., 2012). Also, Hoseini and colleagues (2006) were demonstrated when *Deconex solarsept* used the amount of *pseudomonas aeruginosa*, *staphylococcus aureous*, *mycobacterium*, and *salmonella typhimurium* are controlled(Hoseini et al., 2006).

CONCLUSION

Based on the results of present work, it can be found that the maximum and minimum phenol coefficient are related to *Deconex 53 Plus*and *Saya sept HI*, and consequently, they are the strongest and weakest disinfectants, respectively. The result of MIC antimicrobial test showed that *Saya sept HI* and *Deconex Solarsept* are effective, and also *Saya sept HP* was recognized as the weakest disinfectant. *Staphylococcus aureus* and *Escherichia coli* were more vulnerable strains and *Enterococcus* were more resistance to minimum inhibitory concentration. The MBC test equaled to MIC values. Final disinfection under laboratory condition was performed, and *Saya sept HI* and the *Saya sept HP* was able to control *Pseudomonas aeruginosa*.

CONFLICT OF INTEREST
There Is No Conflict of Interest.

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FINANCIAL DISCLOSURE
None

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