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Original Article

Efficiency of Commonly Used Disinfectants and Antiseptics against Pathogenic Bacteria Isolated from Tripoli University Hospital, Libya

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ABSTRACT

The purpose of this study is to examine the effectiveness of the commonly used disinfectants and antiseptics against a group of nosocomial bacteria isolated from Tripoli University Hospital, Libya. Five bacterial pathogens isolated from different Tripoli hospital departments were obtained in this study are klebsiella spp., Proteus spp., Escherichia coli (E. coli), Acinetobacter spp., Staphylococcus aureus (S. aureus). These isolated bacterial species were sub-cultured and grown in Mueller Hinton Agar (MHA) and subjected to disinfectants and antiseptics impregnated with filter paper discs (disk diffusion assay). The disinfectants and antiseptics used were; Hydrogen peroxide (H_2O_2), OROLIN® Multisept plus (Orolin), Chlorhexidine (CHX), 84 Disinfectant, ACTOSAL® Flache AF (Actosal) and Ethyl alcohol. Our results showed a marked discrepancy in the effect of these antimicrobial agents. The most effective was H_2O_2 antiseptic against the tested bacterial pathogens with inhibition diameters of 10 to 26mm, except for Proteus bacteria which illustrated resistance at high concentrations. Similarly, Orolin disinfectant shows very excellent efficacy against tested bacteria, and even with low concentrations still displayed excellent inhibitory effects among tested pathogenic bacteria. Good efficacy was observed for Actosal disinfectant on all species with diameters of inhibition ranging from 10 to 15mm. S. aureus in particular was very sensitive to 70% Ethyl alcohol (20mm), while all tested species were slightly insensitive to both Ethyl alcohol and CHX. In contrast, no effect of the 84 disinfectant was observed on E. coli, Proteus and Acinetobacter, and was very weak on S. aureus and klebsiella. The results of this study indicated that Orolin disinfectant had comparable effects to H_2O_2 atiseptic but less than to Actosal, 70% Ethyl alcohol and CHX, whereas 84 disinfectant was the weakest one. This study suggests the necessity of applying continuous monitoring to determine the antimicrobial efficacy of these antimicrobial agents regularly. Further studies are required on the consequences of prolonged or misuse of such agents, thus preventing the emergence of disinfectant-induced antimicrobial resistance and antibiotics cross-resistance.

Keywords: Disinfectants, Antiseptics, Tolerance, Resistance, Nosocomial Infections

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الغرض من هذه الدراسة هو فحص مدى فعالية المطهرات والمعقمات الشائعة الاستخدام ضد مجموعة من البكتيريا المعزولة من مستشفى جامعة طرابلس، ليبيا. تم في هذه الدراسة الحصول على خمسة أنواع بكتيرية معزولة من مختلف أقسام مستشفى طرابلس الجامعي الطبي وهي.klebsiella spp، ‏Proteus spp.، E. coli، Acinetobacter spp.، S. aureus تم زراعة هذه الأنواع البكتيرية المعزولة في مولر هينتون أجار (MHA) وتعرضت للمطهرات و المعقمات المشربة بأقراص ورق الترشيح. وكانت المطهرات والمعقمات المستخدمة هي: بيروكسيد الهيدروجين(H2O2) ، OROLIN® Multisept plus(الأورولين)، الكلورهيكسيدين(CHX) ، مطهر 84، ACTOSAL® Flache AF(أكتوسال) والكحول الإيثيلي. أظهرت نتائجنا وجود تناقض ملحوظ في تأثير هذه العوامل المضادة للميكروبات. كان المطهر الأكثر فعالية هو H2O2 ضد البكتيريا المعزولة التي تم اختبارها بأقطار تثبيط تراوحت بين 10 إلى 26 ملم، باستثناء بكتيريا Proteusالتي أظهرت مقاومة عند التراكيز العالية. وبالمثل، يُظهر مطهر الأورولين فعالية ممتازة جدًا ضد البكتيريا التي تم اختبارها، وحتى مع التركيزات المنخفضة لا يزال يظهر تأثيرات مثبطة ممتازة بين البكتيريا المسببة للأمراض التي تم اختبارها. وقد لوحظت فعالية جيدة لمطهر أكتوسال على جميع الأنواع التي تتراوح أقطار تثبيطها من 10 إلى 15 ملم. كانت S. aureus على وجه الخصوص حساسة جدًا للكحول الإيثيلي (20 مم). في حين كانت جميع الأنواع التي تم اختبارها قليلة الحساسة لكل من الكحول الإيثيلي و CHX.في المقابل، لم يلاحظ أي تأثير للمطهر 84 علىE. coli ، Proteusو Acinetobacter وكان ضعيفاً جداً على S. aureus و.klebsiella. أشارت نتائج هذه الدراسة إلى أن مطهر الأورولين كان له تأثيرات مماثلة لمطهر H2O2 ولكن أقل من أكتوسال والكحول الإيثيلي وCHX، في حين كان مطهر 84 هو الأضعف. تشير هذه الدراسة إلى ضرورة تطبيق المراقبة المستمرة لتحديد فعالية مضادات الميكروبات لهذه العوامل المضادة للميكروبات بانتظام. هناك حاجة إلى مزيد من الدراسات حول عواقب الاستخدام المطول أو سوء استخدام هذه العوامل، وبالتالي منع ظهور مقاومة مضادات الميكروبات الناجمة عن المطهرات والمقاومة المتبادلة للمضادات الحيوية

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INTRODUCTION

Antibiotic-resistant bacteria are becoming more common sources of hospital-acquired infections. This has led to high rates of morbidity and mortality among patients, which significantly raises the cost of care due to longer hospital stays and the requirement for more expensive medications [2,1]. For instance, methicillin-resistant *Staphylococcus aureus* (MRSA) is the main cause of nosocomial infections that are becoming increasingly difficult to combat due to developed resistance to all classes of present antibiotics [3]. It has been postulated that countries with low incidence of MRSA infections tend to use stronger antibiotic restriction polices and implement more stringent infection control protocols [2,4]. Nowadays, novel antimicrobial agents are desperately needed because those currently available have become ineffective due to the emergence of antibiotic resistance in hospitals and communities [5]. One way to eliminate the global spread of antibiotic resistance and control the circulation of resistant bacteria in hospitals, homes and communities is the constant use of disinfectants and antiseptics [6]. These substances are antimicrobial slowing or killing germs whether if presented on a non-living object (disinfectants) or on the living tissues (antiseptics) and the main reason for using such substances is to reduce exposure to infection. However, with longer use the germs might also have acquired resistance to disinfectants of various kinds [7]. In recent years, numerous publications have demonstrated that bacteria can develop resistance not only to antibiotics but also tolerance to sanitizers and disinfectants [8-10]. In fact, these substances are widely used in hospitals and other healthcare centers to control microbial growth in living tissues and non-living objects and are considered an essential part of infection control practices and help prevent hospital infections [11].

Alcohols are one of the most important sanitizing antimicrobial agents that can denature proteins, meaning that, gives excellent bactericidal efficacy. Alcohol is essential in medical detergents and disinfectants and becoming an alternative to hand washing in healthcare settings and some public places due to its effectiveness in the rapid destruction of some pathogens [12]. Remarkably, substantial evidence exists supporting the emergence of antimicrobial-resistant bacterial isolates. For example, a study conducted by Pidot *et al*., 2018 showed that *Enterococcus faecium* isolates were tolerant to isopropanol alcohol [13]. Furthermore, Akın and co-workers have detected resistant isolates against 3% H_2O_2 disinfectant [14]. In addition, a recent study by Morante *et al*., 2021 elucidated resistance to CHX and isopropanol alcohol among *Klebsiella pneumoniae* isolates found in Peru hospitals [15]. Thus, over the past few years, there has been an increased concern due to the misuse of these disinfectants and antiseptics worldwide which as a consequence might cause bacteria to develop resistance [16].

Currently, a new concern from alcohol-tolerant bacteria has started to be developed [17]. In a previous study, researchers examined the alcohol tolerance of 139 hospital *E. faecium* isolates collected from 1997 to 2015 from two Melbourne hospitals and examined how well these isolates survived when treated with dilute isopropyl alcohol. Interestingly, they found that samples collected after 2010 were more tolerant to 70% isopropanol surface disinfection compared to isolates collected before 2010 [13]. Similarly, another study demonstrated the inhibitory effects of five common antiseptics; CHX, H_2O_2, Iodine, Ethanol, and Dettol on MRSA, *Acinetobacter baumannii*, *E. coli*, and *klebsiella* species, and significantly found that all tested bacteria were resistant to ethanol at all concentrations. Indeed, this study also showed that H_2O_2 was the most effective disinfectant compared to other disinfectants followed by CHX [18].

It could be argued that either due to overuse or misuse of disinfectants and consumption of low-quality products, tolerance or even resistance against these disinfectants can occur [19, 20]. Consequently, as a result of disinfectants possibly being no longer as effective as should be, this would seriously threaten clinical settings and patient safety [21]. The aim of this study is to examine the effectiveness of commonly used disinfectants and antiseptics in Tripoli university hospital against the most common nosocomial bacteria and determine if the used concentrations at these healthcare facilities are capable or not of eliminating the superbug.

METHODS

Microbial strains

Five strains of pathogenic bacteria were used, of which one was Gram-positive *Staphylococcus aureus* (*S. aureus*), and four were Gram-negative *klebsiella* spp., *Proteus* spp., *Escherichia coli* (*E. coli*), *Acinetobacter* spp. All bacteria were obtained from the different departments at Tripoli University Hospital and identified by standard microbiological tests. These isolates were used to evaluate the inhibitory activity of disinfectants and antiseptics against them.

Culture preparation and susceptibility test

A swab was taken from a colony of bacteria using a sterile cotton swab dipped in normal saline, and the swab was distributed over the entire Muller-Hinton dish. Cultivate bacteria was tested for the effect of antiseptics and sterilizers using the (Disc Diffusion Method). The selected disinfectants or antiseptics were impregnated with filter paper discs with a diameter of (6mm) saturated with the specific concentrations and used separately. Then sterile forceps with a pointed end were used to transfer the discs to the surface of the implanted dish and lightly pressed the surface to fix it on the culture medium. To avoid overlapping the areas of inhibition, an appropriate distance was left between one disc and another. Then all dishes were placed in the incubator for 24 hours at 37°C. After the incubation period, the diameter of the bacterial growth inhibition zones was measured around the discs, which appear as a halo without bacterial growth surrounding the disinfectant disc.

Disinfectants and antiseptics

Hydrogen peroxide (H_2O_2), OROLIN® Multisept plus (Orolin), Chlorhexidine (CHX), 84 Disinfectant, ACTOSAL® Flache AF (Actosal) and Ethyl alcohol were used in this study to test their efficiency in eliminating bacteria and examine the sensitivity of bacteria to such compounds. These antimicrobial substances are commonly used in Tripoli University Hospital and were prepared according to manufacturer recommendations. The manufacturer and other information of such substances are shown in Table 1.

As recommended by the manufacturer, several increasing effective concentrations of these substances were prepared using the dilution methods to test in this study. Furthermore, some particular concentrations recommended by the hospital were also examined (Table 1). A dilution procedure was done (1:100) where the original concentration of the substance was considered 100%, and the subsequent concentration was prepared by adding 1 ml of disinfectant in a tube with 99 ml of distilled water to give a concentration of 1% and so on. Depending on the type of disinfectants or antiseptics used in this study, different concentrations were applied in this study (Table 1).

Table 1: Types and concentration of disinfectants and antiseptics used in the study.

Trade name	R/Con.	H/Con.	Main effective ingredient	Manufacture
Hydrogen Peroxide (H_2O_2)	3%, 6%	3%, 6%	Hydrogen Peroxide (H_2O_2)	(AQUA) Turkey
OROLIN® Multisept plus	2%	2%, 5%, 10%, 15%	• Didecyldimethylammonium chloride >2.5% – <5% • N-(3-Aminopropyl)-N-dodecylpropane -1,3-diamine >5% – <15% • 2-amino-2-methylpropanol >2.5% – <5%	(OCC) Switzerland
Chlorhexidine (CHX)	100%	100%	• Chlorhexidine 0.12% • Cetylpyridinium chloride 0.05%	(DENTAID) Spain
84 Disinfectant	1%	1%, 2%, 5%	Parachlormetaxylenol 4.5% - 6.5%	(GIG) Germany
ACTOSAL® Flache AF	100%	100%	• Poly(oxy-1,2-ethanediyl), alpha-(2-propylheptyl)-omegahydroxy > 9% – < 11% • Didecyldimethylammonium chloride > 7% – < 9% • Polyhexamethylene Biguanide Hydrochlorid > 2% – < 3%	(ACTO) Germany
ETHANOL	70%	70%	Ethyl alcohol	(WAN CARE) Turkey

RESULTS

In order to examine the efficiency of using different concentrations of Orolin disinfectant as recommended by Tripoli university hospital (Table 1) against our bacteria isolates 2%, 5%, 10%, and 15% of disinfectant concentrations were prepared and impregnated into paper discs and exposed to bacterial isolates as described in material and methods. As shown in Figure 1, Table 2 and Figure 3A, our results of the disc diffusion method showed a sensitivity of tested microorganisms to disinfectant Orolin at different concentrations which were compared to the antiseptic alcohol at a concentration of 95% as a control. These results revealed a clear inhibitory activity of Orolin disinfectant at selected concentrations against all types of bacteria and were apparent at the highest concentration (15%) for most of the bacterial strains suggesting that all isolates exhibited excellent sensitivity to Orolin. The sensitivity to 95% alcohol was very limited due to alcohol evaporation over time, (Figure 1).

To test the growth rate of our bacterial isolates against all disinfectants and antiseptics listed in Table 1 Table 1, recommended concentrations whether from the hospital or from the manufacturer were applied in this study. The results showed that the five types of bacteria isolates (*Klebsiella* spp., *Proteus* spp., *E. coli*, *Acinetobacter* spp., and *S. aureus*) were sensitive to H_2O_2 at both used concentrations (3% & 6%) with inhibition diameters ranging between 10-26mm except for the *Proteus* spp. which shows resistance at 6% concentration (Table 2 and Figure 3C). Interestingly, despite *E. coli* bacteria having clear sensitivity to most disinfectants, it shows a clear resistance to 84 disinfectants at all given concentrations (Table 2 and Figure 3B). Similarly, *Acinetobacter* spp., *Proteus* spp. illustrated resistance to 84 disinfectants at 2% and 5% concentration (Table 2 and Figure 3B), suggesting a possible emerging resistance against 84 Disinfectant. Although *S. aureus* demonstrated tolerance against 84 disinfectants at concentration of 2% and 5% with small zone of

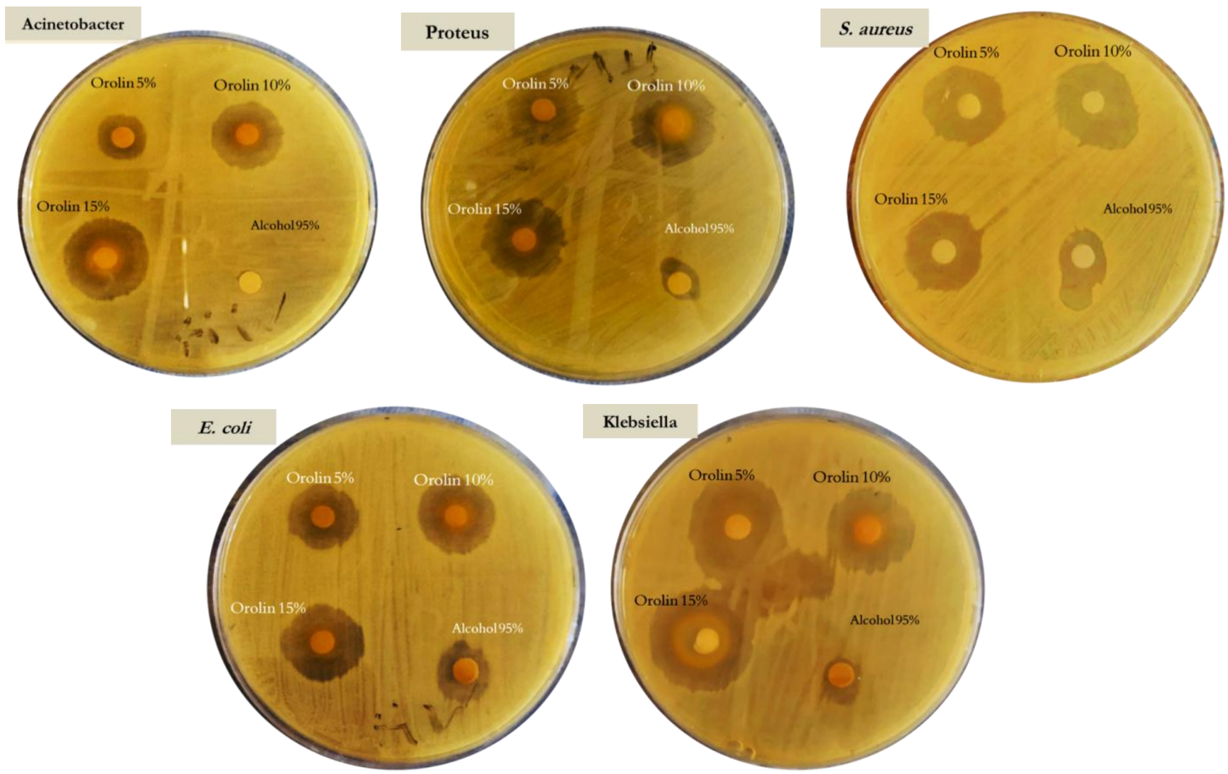

Figure 1: Inhibition zones caused by Orolin at selected concentrations of 5%,10% as well as 15% and alcohol at 95% on the studied bacteria.

Table 2: The distribution of bacteria sensitivity against disinfectant concentrations, the numbers represent the diameters of the inhibition zone (mm).

		Antiseptics and disinfectant concentrations											
		Orolin 2%	Orolin 5%	Orolin 10%	Orolin 15%	84 D 1%	84 D 2%	84 D 5%	Ethyl 70%	CHX	Acto AF	H_2O_2 3%	H_2O_2 6%
Tested Bacteria	Acinetobacter	16	12	18	22	6.5	R	R	8	9	10	15	18
	Proteus	16	17	20	20	6.5	R	R	7	6.5	12	10	R
	S. aureus	22	20	20	20	R	8	9	20	13	14.5	26	18
	E. coli	14	17	19	20	R	R	R	14	9	15	18	20
	Klebsiella	10	22	20	25	6.5	6.5	6.5	10	12	15	17	18

Oro = Orolin Multisept Plus / **84** = 84 Disinfectant / **Ethyl** = Ethyl alcohol / **CHX** = Chlorhexidine / **Acto** = Actosal Flache AF / **H_2O_2** = Hydrogen Peroxide. The yellow shading indicates resistance. Grey shading shows maximum sensitivity.

inhibition ranging between 8 to 9mm, its resistance at the lowest concentration was detectable (Table 2). On the other hand, *Klebsiella* bacteria were more sensitive to all disinfectants and recorded zone of inhibition ranged between 6.5-25mm.

Orolin disinfectant was very effective against all bacteria tested and its inhibitory effect was increasing at higher concentrations followed by H_2O_2. Conversely, CHX had comparable effects to Actosal but less than ethyl alcohol (Table 2). *S. aureus* species in particular represented a maximum zone of inhibition against 3% H_2O_2 (26mm) compared to the other tested disinfectants (Table 2 and Figure 2). In support of these results, it is clear as shown in Figure 3 that 84 Disinfectant was the least effective for all types of bacteria, followed by 70% Ethyl alcohol and CHX. Our results showed that Orolin was the most effective disinfectant than the others followed by H_2O_2 (Figure 3A and C).

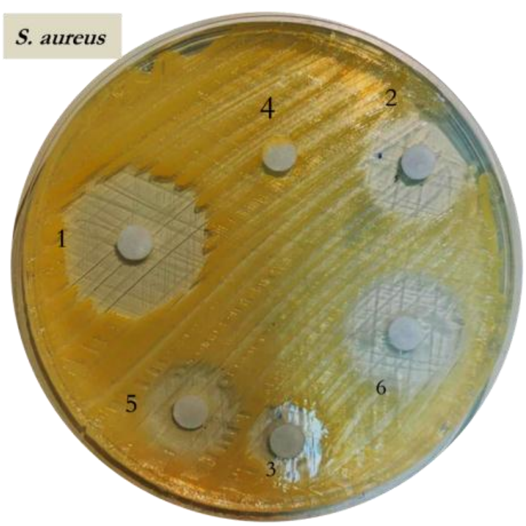

Figure 2: Inhibition growth zone test of disinfectants and antiseptics used in the study toward S. aureus. H_2O_2 3% [1], 10% Orolin [2], 100% CHX [3], 84 Disinfectant 1% [4], 100% Actosal [5] and 70% Ethyl alcohol [6].

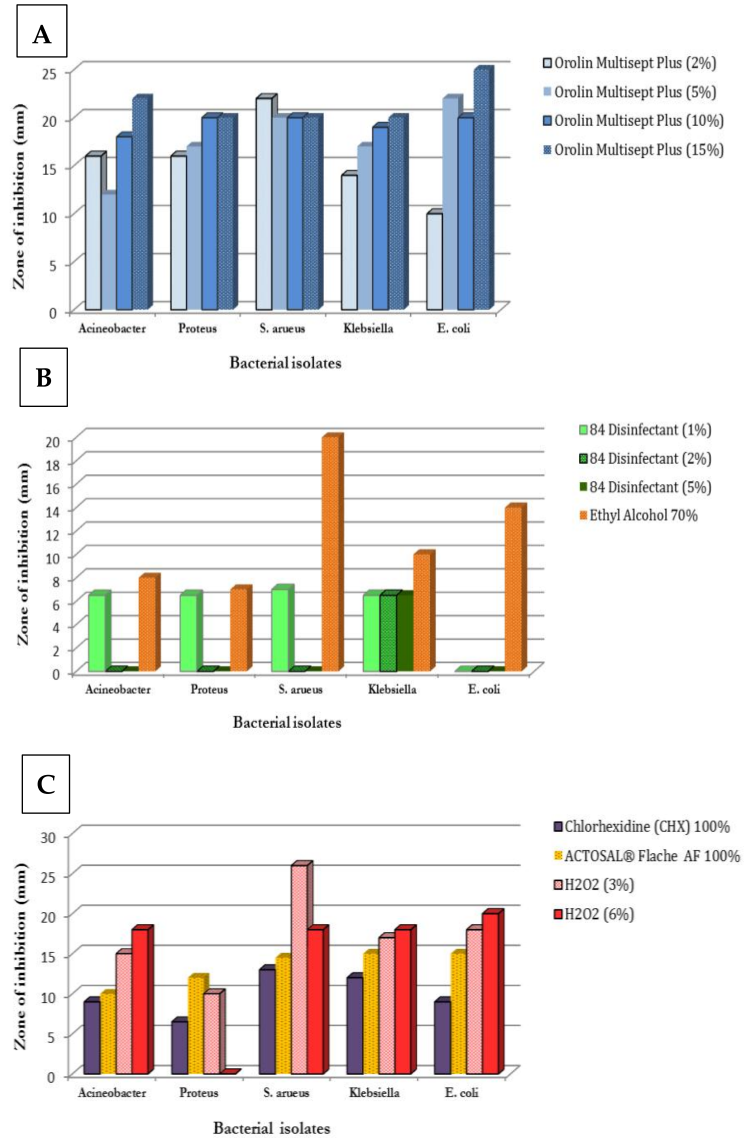

Figure 3: Distribution of bacteria sensitivity against disinfectant concentrations. A is the distribution of bacteria sensitivity against Orolin and 84 Disinfectant disinfectants. B is the distribution of bacteria sensitivity against Ethyl alcohol. C is for CHX, Actosal and H_2O_2.

DISSCUSION

The isolated bacteria obtained in this study were subjected to susceptibility tests and exposed to several disinfectants and antiseptics used to control microbial infection in Tripoli University hospitals. The purpose of this test was to evaluate the inhibitory effect of these antimicrobial compounds.

Clearly, the five tested types of bacteria used (*Klebsiella* spp., *Proteus* spp., *E. coli*, *Acinetobacter* spp., and *S. aureus*) were sensitive to the H_2O_2 at a concentration of (3% and 6%) produced zone of inhibition, ranging between 10 mm to 26mm, except that *Proteus* bacteria was capable to resist this compound at a concentration of 6% (Table 2). In agreement with this, an observation was conducted by Hassanain *et al*., 2019, concluding that H_2O_2 was the most effective antiseptic used in the study as demonstrated excellent inhibitory effects on all tested nosocomial bacteria even with lower concentrations [18]. Similarly, in support of this finding Lineback and co-workers found that H_2O_2 antiseptic had significantly higher bactericidal efficacies when compared with other disinfectants [22]. It was envisaged that this action of H_2O_2 is due to a potent oxidant that produces hydroxyl radicals which as a consequence will perturb the cell membrane, lipids and other essential cell components [18,23].

On the other hand, Orolin disinfectant was shown to be the strongest disinfectant used, and even that at the lowest concentrations it still had excellent inhibitory effects among tested bacteria (Table 2). Orolin is a powerful disinfectant due to its distinctive composition includes strong corrosion inhibitors [24]. Obviously, Orolin gives inhibitory zones ranged between (10-22mm) at 2% concentration and increased at 15% concentration to reach between (20-25mm), which demonstrates a growth decline in a disinfectant dose-dependent manner (Table 2, Figures 1 and Figure 3A).

Unlike Orolin disinfectant, 84 disinfectants did not produce any significant zones of inhibition against the tested organisms even at increasing concentrations (Table 2). In fact, 84 disinfectant was ineffective against the tested bacteria with comparable zone sizes to other used disinfectants (Figure 3B). Surprisingly, the most tested bacteria (*Acinetobacter* spp, *Proteus* spp. and *E. coli*) were resistant to this particular disinfectant and both *S. aureus* and *Klebsiella* spp. illustrated tolerance, suggesting that the manufacturer should take this into consideration (Table 2 Figure 2). 84 disinfectant is sodium hypochlorite containing 5.5% to 6.5% chlorine and resistance against this compound was indeed documented [25, 26]. It was thus predicted that the dramatic increase in the use of this disinfectant during the COVID-19 epidemic has a key role in developing bacterial resistance in clinical settings [25].

Actosal is widely used as a surface disinfectant in hospitals and contains mainly diethyldimethy-lammonium chloride (quaternary ammonium compound commonly applied for cleaning and disinfection purposes) [27]. In this study, Actosal demonstrated decent efficacy against the clinical isolates with diameters of inhibition ranging from 10 to 15mm (Table 2), although reduced susceptibility to diethyl dimethylammonium chloride was reported [16,22,28]. Alternatively, 70% Ethyl alcohol revealed a quite variation in zones of inhibition against the tested organisms. We noticed that it showed good activity against *S. aureus* (20mm) indicating susceptibility, while a moderate effect was evident in both *E. coli* and *Klebsiella* bacteria with diameters ranging between (10-14mm) (Table 2). Nevertheless, both *Proteus* and *Acinetobacter* species were Ethyl alcohol-tolerant bacteria (Table 2). Finally, a lower level of growth activity was detected in the studied bacteria when treated with the oral CHX antiseptic which exhibited a zone of inhibition in the range of 6.5-13mm (Table 2 and Figure 3C). CHX is also used in hospital settings such as in cleaning surfaces and surgical sites but is mainly applied in dental practice. Interestingly, numerous recent publications have indicated the emergence of resistance to CHX [15,29,30,31]. In general, the results of this study indicated that H_2O_2 had comparable effects to Orolin disinfectant but less than to Actosal, Ethyl alcohol and CHX.

While there is a good understanding of antibiotic resistance, tolerance to disinfectants remains unclear and yet under investigation. Unlike antibiotics, disinfectants mode of action can be nonspecific, targeting different mechanisms or sites in bacterial cells which is subsequently imposing cell disruption. Thereby, conferring resistance is unlikely [32,33]. Conversely, tolerance or even resistance against overuse or misuse disinfectants can be acquired by a number of mechanisms such as; (i) developing mutation of an endogenous chromosomal gene, (ii) incorporating mobile genetic elements such as plasmids, jumping transposons and other cell elements, and (iii) due to changes in cell membrane permeability or increased efflux pump expression [16,20]. In addition, a threaten linkage between long term usage of disinfectants and emergence of resistance to antibiotics was recently reported [34]. This is particularly a matter of concern since available data on this situation here in Libya is scarce. At present, regulations for antibiotic consumption worldwide are stringent. Disinfectants are however subject to fewer restrictions compared to antibiotics. Therefore, it is imperative to call for the controlled use of disinfectants to manage the rise of antibiotic resistance caused by prolonged exposure to these substances [34].

CONCLUSION

This study confirms the efficient ability of both Orolin disinfectant and H_2O_2 antiseptic to degrade all isolated bacterial species followed by Actosal and 70% Ethyl alcohol. However, 84 disinfectant was the weakest one and showed no significant effects on these species. Collectively, this evidence suggests the urgent need for continuous surveillance to determine the antimicrobial efficiency of disinfectants and antiseptics regularly. We recommend a daily review of the effectiveness of disinfectants and chemical sterilizers against multidrug-resistant pathogens is deemed necessary. Further comprehensive studies are required to investigate this issue by collecting larger samples from different hospitals and healthcare centers and using a broad collection of antimicrobial agents to determine whether the widespread use of these products is responsible for the development of microbial resistance or not.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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