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Study of the Efficacy of Disinfectant against Bacterial Contamination in Burns Unit –Algumhory and International Yemen Hospitals in Taiz City

Abdu M. Alkolaibe[a], Gamal A. AL-Ameri[b], Mawhoob N. Alkadasi[c], Abdubaset A.Zaid[d]

[a]Department of Microbiology Science collage Taiz University
[b]Department of Microbiology Medical collage Taiz University
[c]Department of Chemistry , Zabeed Education Collage, Hudaiadah University
[d]Post Graduate and Research Centre, Maulana Azad College, Aurangabad, India.
abdulbasitalzazai4@gmail.com

Abstract:

Aim: *To evaluate the bacteriological examination of burn wound infection and their susceptibility patterns to commonly used disinfectants.*

Methodology: *This study was carried out on 60 pus swabs obtained from 60 burn wound patients at burn's Center in Al-gmhori and International Yemen hospitals located in Taiz city. The study period was March 1st, 2014 to August 29, 2014. The age of the studied burn patients ranged from one months10 to above 40 years.The antimicrobial activity of disinfectant and antiseptic was tested against 4 types of bacteria: P.aeruginosa, E.coli, S.aurus and proteus spp., in which bacteria tested for disinfectants and antiseptics were isolated from burns and wound infections from hospital environment. Disinfectants and antiseptics sensitivity was carried out by using Muller-Hinton medium, 5-10 colonies of each isolate were picked up with sterile loop and suspended into 2.5ml of sterile distilled water, suspension was taken by a sterile cotton swab then streaked the surface of all the plate in three different planes. By using the sterile forceps, the disks were placed on the inoculated plates and pressed firmly but gently into surface of the agar and then incubated at 37oC for 18-24 hrs. After incubation the diameter of complete inhibition zones were measured using reflected light and ruler.*

Results: *In the present study found that 42 patient were having positive specific bacterial growth with percentage of (70%),when the studied patients classified to their sex it was found that: (26 males) and sixteen (16 females), the frequency of males was (43%) and (27%) females and the predominant positive result found among patient age 21-40 with percentage of (40.5%). Among the 42 patients who has positive bacterial infection the highest positive result was found in the age group of 21 to 40 years (40.5%), follow by age group over 40 years (35.7%) and the least age group found in the age <20 years (23.8%). In the present study found that Pseudomonas aeruginosa (20%) was the predominant isolate Staphylococcus aureus (35.7%) was the predominant isolate, second most wereEscherichia coli(23.8%), third one was Staphylococcus aureus (24%) and lowest percentage was recorded by Proteus sp. (19.1%). The present study showed that effective antiseptics tested against isolated bacteria were chlorhexidine (Salvon) and formalin respectively, secondly Dettol recorded low activity, other selected disinfectants showed non-significant activity. In the present study E. coli and Proteus sp. were found to be the most susceptible bacteria being tested in this study against disinfectants and antiseptics while Pseudomona aueroginosa, Staphylococcus aureus were the most resistant bacteria to disinfectants agents*

Keywords: *Study of the efficacy of disinfectant against bacterial contamination in burns Taiz Yemen*

1. INTRODUCTION

Wound infection has been defined as wound with pus visible to the necked eye, whether or not organisms could be cultured from the purulent material [1]. Open injuries whether caused a laceration, a crash injury or a penetrating missile wound have three facets in common and differ only in a matter of degree. All are considered to be primarily contaminated by microorganisms,

all may contain foreign babies and all are likely to contain a significant amount of devitalized or necrotic tissue [2]. The most likely organisms to infect clean operation wounds in hospital are Staph. Aureus, ps. aerugionsa, and E.coli, as with accidental wounds local treatment often sufficient [3]. If infection is deep- seated or becomes generalized appropriate systemic treatment must be administered [4].

In addition, the entry site should be cleansed daily and treated with one of the antiseptics such ascentaulon, Hibitane, and quinolines [5].

Disinfectants are agents that destroy or inhibit the growth of microorganisms in or on living tissue while disinfectant are similar but are used on inanimate objects or surface [6].These agents such as alcohols, phenols, iodine and chlorine were used extensively in hospitals and other health care settings for infections control and prevention of nosocomial infections [7]. An ideal disinfectant to overcome the antimicrobial resistant pathogens should have broad spectrum of antimicrobial activity [8] and the efficacy of these agents may be affected by PH, detergent base, temperature, organic matter, ionic and type of the surfactants [9]. Mechanisms of action of biocides on whatever type of microbial cell can be defined as the interaction of antiseptic or disinfectant with the cell surface followed by penetration into the cell and action at the target site[10]. In addition, the interaction at the cell surface can produce a significant effect on viability [11] but most antimicrobial agents appear to be active intracellularly [12]. The wide spread use of these agents has promoted some speculation on the development of microbial resistance[13] and this resistance to disinfectants and antiseptics mainly intrinsic in nature whereas antimicrobial resistance is frequently conferred by plasmid or transposons, which have allowed rapid and extensive spread through the globe. Development of resistance to antimicrobial agents and biocides is particularly warning problem which is compounded by cross–resistances mechanisms (between antibiotic and between antibiotic and biocide) that may exist in certain bacteria such as pathogenic strains of E.coli [14]. Among bacteria, biocide sensitivity is based on the permeability of the biocide through the cell wall [15] and impermeability is influenced by the composition of cell wall and physiologic adaptation of the microorganisms to it's environment [16].Gram–negative bacteria are generally less susceptible to biocides because of their complex cell wall [17] in which the outer membrane of Gram–bacteria act as permeability barrier in limiting or prevention the entry of many chemically unrelated types of antibacterial compounds.Germicides have multiple target site for their cidal effects on microorganisms while antibiotic have single target site[18]. It will be continued requirement for new and potent antimicrobial agents together with techniques suitable for control and destruction of microbial pathogens[19].Disinfectants are usually used in dilutions, however it has been shown that when some of these agents are diluted for use, some Gram negative bacteria e.g. Pseudomonas aeruginosa can still survive making them ineffective against nosocomical infections [20, 21]. The emergence of resistant microorganisms in hospitals and the community is causing problems for both the treatment of patients and infection control. Organisms of particular concern include methicillin- resistant Staphylococcus aureus, glycopeptide resistant enterococci and extended spectrum beta-lactamase producing Klebsiella [22]

All these organisms are transferred from patient to patient on staff hands. A recent major review of antibiotic resistance emphasized the importance of hospital infection control, and the control of these organisms, and many authorities have reiterated the key role of hand washing with disinfectants [23]. A vast amount of work has done over the post 50 years in attempts to explain the infection that can follow “clean” surgical operation, but still no complete satisfactory solution (s) has been documented. There is still uncertainly as to how often a wound is infected in the operating room, and how often at a later data during the healing of the wound.

There are those who deny that air in the operating theatre is an important source of infection because bacterial pathogens from only a minute fraction of colonies grown from the air. In the ward, on the other hand, the aerial route for post-operative cross-infection is regarded as a potent one [24, 25].

The aim of this study to evaluate the bacteriological examination of burn wound infection and their susceptibility patterns to commonly used disinfectants and antiseptics

2. MATERIAL AND METHODS

2.1. Study Population

This study was carried out on 60 pus swabs obtained from 60 burn wound patients at burn's Center in Al-gmhori Hospital located in Taiz city. The study period was March 1st, 2014 to August 29, 2014. The age of the studied burn patients ranged from one months10 to above 40 years.

2.2. Sample collection

All swabs obtained were cultured directly on blood agar and MacConkey agar for isolation of aerobic bacteria. Cultured plates were examined after overnight incubation at 37oC, if no growth obtained on plates they were re-incubated for another 24 hrs. [26]. Identification of pathogenic bacteria was based on gram stained smear, biochemical test and culture media [27].

2.3. Disinfectants and Antiseptics

Different types of disinfectants and antiseptics as shown in table (1) are used to test susceptibility of bacteria.

Table 1. *Disinfectants and Antiseptics Used in this Study*

Scientific name	Ethanol	Iodine	Chlorhexidine, cetrimide	Formaldehyde	Choroxylenol	sodium hypochlorite	Methyl alcohol
Traditional name	Etheyl alcohol	Betadine	Salvon	Formalin	Detol	Chlorox	Spirit

2.4. Sterilization Test of Disinfectants and Antiseptics

The chemical disinfectants and antiseptics being used in this study were tested for their sterility from microorganisms for accurate susceptibility test as follow. 0.10 ml of the disinfectants and antiseptics was added onto blood agar medium and was spread by spreading method. The plate incubated under aerobic condition at 37Cº for 7 days [28].

2.5. Disinfectants and Antiseptics Susceptibility Test

The antimicrobial activity of disinfectant and antiseptic was tested against 4 types of bacteria: P.aeruginosa, E.coli, S.aurus and proteus spp., in which bacteria tested for disinfectants and antiseptics were isolated from burns and wound infections from hospital environment. Disinfectants and antiseptics sensitivity was carried out by using Muller-Hinton medium, 5-10 colonies of each isolate were picked up with sterile loop and suspended into 2.5ml of sterile distilled water, suspension was taken by a sterile cotton swab then streaked the surface of all the plate in three different planes. By using the sterile forceps, the disks were placed on the inoculated plates and pressed firmly but gently into surface of the agar and then incubated at 37oC for 18-24 hrs. After incubation the diameter of complete inhibition zones were measured using reflected light and ruler [29].

2.6. Result

In the present study, 60 patients male and female who were selected randomly from burn unit in Algumhory and international Yemen hospital at Taiz city , all of them undergo for pus ,skin scales culture to identify and survey for the most bacterium cause nosocomial infection among those kind of resident patient. In the current study found that 42 patient were having positive specific bacterial growth with percentage of (70%) while 18 patient have negative with percentage (30%). Among positive bacterial growth male shown predominant infection with 26(61.9%), while in female it was 16(38.1%). as shown in table 2.

Table2. *Distribution of infection according to sex*

SEX	**No of male pt**	**infected**	**%**	**Non infected**	**%**
Males	39	26	43	13	22
females	21	16	27	5	8
Total	60	42	70	18	30

Among the 42 patients who has positive bacterial infection the highest positive result was found in the age group of 21 to 40 years (40.5%), follow by age group over 40 years (35.7%) and the least age group found in the age <20 years (23.8%) as shown in table3.

Table 3. *Classification of infection according to age*

Age	**No of pt**	**No growth**	**infected**	**% Infected pt**
Less than 20	12	2	10	23.8
21-40	31	11	17	40.5
More than 40	17	5	15	35.7
Total	60	18	42	100

In this study,the predominantly isolated microorganisms were *Psudomonus aueroginosa*(35.7%), *E. coli* (23.8%) *Staphylococcus aureus*, (21.4%) and *proteus sp*.(19.1%) as shown table 4.

Table 4. *Distribution of Bacterial Infection Among Patients Samples*

Type of bacterium	*E. coli*	*%*	*Staphau reus*	*%*	*Pseudomonas aueruginosa*	*%*	Proteus sp	%	Total positive	Total %
No of positive growth	10	23.8	9	21.4	15	35.7	8	19.1	42	100

To fulfill our work we decide to examine the efficacy of some disinfectants against the bacteria isolates from our studied patients by preparing some dilution of traditional disinfectants like, Ethanol, Iodine, Chlorhexidine, Formaldehyde, Choroxylenol, sodium hypochlorite and spirit as shown table5. From concept and principle of bacterial antibiotic sensitivity test we get the following results which then demonstrate the sensitive and resistant against isolated bacteria table5.

Table5. *concentrations of disinfectant are used susceptibility test.*

Traditional ,comm.. name		**Scientific name**	**Conc.%**
1	Ethyl alcohol	Ethanol	5%
2	Betadine	Iodine	
3	Salvon	Chlorhexidine, cetrimide	1.50%
4	formalin	Formaldehyde	39%
5	Detol	Choroxylenol	non
6	Chlorox	sodium hypochlorite	5%
7	Spirit	Methyl alcohol	70%

The present study showed that effective antiseptics tested against isolated bacteria were chlorhexidine (Salvon) and Formaldehyde (formalin) respectively, secondly Dettol recorded low activity, other selected disinfectants showed non-significant activity. In the present study, *E. coli and Proteus sp.* were found to be the most susceptible bacteria being tested in this study against Detol, Salvon and formalin disinfectants and antiseptics while more resistant to Chlorox and Spirit.In current study also found that *Staph.aureus* showed more sensitive to Salvon and Chlorox while more resistant to Detol, formalin, Spirit, Iodine and Ethanol in all hospitals. Moreover *Pseudomona aueroginosa*, was resistant bacteria to Chlorox, formalin, Spirit, Iodine and Ethanol while sensitive to Salvon and Detol disinfectants agents in all hospitals as shown in table 6.

Table 6. *Susceptibility of Isolated Bacteria from Disinfectants*

Type of bacteria		***Salvon***	***Ethanol***	***Iodine***	***Spirit***	***fromalin***	***Detol***	***Chlorox***
Staph.aureus	*1*	*S++*	***R***	*R*	*R*	*R*	*R*	*S++*
E. coli	*2*	*S++*	***S***	*R*	*R*	*S*	*S*	*R*
Psudomonas .sp	*3*	*S++*	***R***	*R*	*R*	*R*	*S*	*R*
Proteus sp	*4*	*S*	***R***	*S++*	*R*	*S*	*S++*	*S*

3. DISCUSSION

Despite best efforts to identify and eliminate infectious microorganisms, they continue to emerge and re-emerge. These pathogenic bacteria significantly contribute to human illness and death especially as a result of hospital acquired infection/s [30].

One of the goals of disinfection in hospitals is to reduce the risk of nosocomial infection in patients. A great number of disinfectants are used in healthcare settings, including glutaraldehyde, formaldehyde and chlorine releasing agents and compounds. These agents are considered gennicidal when recommended and used in appropriate concentrations for cleaning patient-care items and instruments [31].

In the current study found that 42 patient were having positive specific bacterial growth with percentage of (70%) while 18 patient have negative with percentage (30%). Among positive bacterial growth male shown predominant infection with 26(61.9%), while in female it was 16(38.1%). This result was in agreement with the finding reported by reported by Ghaffar et al, who found that burn wound infection in males was 1447 (64.4%) while burn wound infection in females were 799 (35.6%) [32]. In a similar study, showed that burn infection in females (60%) was more than male (40%) in India [33]. In contrast to Rajupt et al., showed that burn infection in females (60%) was more than male (40%) in India [34]. Other study reported and found that burn wound infection in males 120 (59.1%) was more than burn wound infection in females 83 (40.9%) [35].

In this study, it was found that the highest distribution of burn wound infection found within the age group 21-40years 17 (40.5%) result shown in table 3. This result was in agreement with Kwong and Chung, found that the age group 19-40 years 23 (55%) were more susceptible to burn wound infection than other age groups [36].. In contrast reported have done by Shakibaie et al., who found that the age group 10-19 years was more susceptible to burn wound infection than other age groups [37].

In this study, the predominantly isolated microorganisms were Psudomonus aueroginosa (35.7%), E. coli (23.8%) Staphylococcus aureus, (21.4%) and proteus sp.(19.1%). Similar findings of the predominance of these bacteria in disinfectant and antiseptic solutions and the different strains exhibiting variable resistance to disinfectants and antiseptics have been reported earlier [38]. Other strains like Pseudomonas mirabilis and E. coli have also been isolated and linked to nosocomial outbreaks [39]. Amongst the major contaminants of their disinfectants, Tytler et al. [40] reported that Gram-negative bacteria constituted 69% of the microbial contaminants and E. coli remains the most predominant one.

The antimicrobial properties of dettol, Ethanol, Iodine, Salvon, Chlorox and Spirit have been described by several authors including Drexler [41]. The mechanism of action of disinfectant or antiseptic on the microorganism remains the same irrespective of the type and is exerted through the penetration into the cell and action at the target site(s). The latter can produce a significant effect on the viability as most of the biocides appear to act through intra-cellular mechanism [42].The sensitivity or resistance at the level of the bacterial cell membrane, therefore, can be very important factor in determining the final outcome of the treatment with the proposed disinfectant in the hospital practice. Some of these disinfectants also work by production of destructive chemicals against various pathogenic bacterialto attack membrane lipids, DNA and other essential cell components [31].

The effectiveness of disinfectants in controlling nosocomial infection is often compromised by the fact that many of the disinfectants used in hospitals have been reported to be contaminated with organisms during the preparation processes [43].

chlorhexidine (Salvon) and formalin were the most potent and effective disinfectants according to this study followed by Dettol which exhibited lower activity while Ethanol, Iodine, Chlorox and Spirit showed non-significant activity. According to the results obtained by Fakhriddeen, [44]. Chlorohexidine cetramid was the potent disinfectants against bacteria [44]. He arranged the disinfectants according to their potency as chlorohexidine cetramid, CHX, PVP-I, chloroxylenol, formaldehyde and H2O2.

In the present study E. coli and Proteus sp. were found to be the most susceptible bacteria being tested in this study against disinfectants and antiseptics while Pseudomona aueroginosa, Staphylococcus aureus were the most resistant bacteria to disinfectants agents.Due to the capacity of surviving in unfavorable environmental conditions and its high resistance to antibiotic agents, antiseptics and disinfectants, Pseudomonas aeruginosa and Staphylococcus aureus continues to be

an important pathogen in hospital acquired infections, mainly respiratory and urinary infections Olowe et [45]. Fernandez-Astorga et al. [46] reported that the high resistance of Pseudomonas spp. to cationic agents seems to be associated with the chemical composition of their external membrane. This study also demonstrated that Pseudomonas aeruginosa was a problem to the antibiotics, as well as the disinfectants tested.

It is clear that microorganisms can adapt to a variety of environmental, physical and chemical conditions, and therefore not surprising that resistance to extensively used antiseptics and disinfectants has been reported. Many of these reports of resistance has arisen due to inadequate cleaning, incorrect product use and ineffective infection control practices which cannot be underestimated. With growing concerns about the development of biocidal resistance and cross-resistance with antibiotics, clinical isolates should be under continual survcillancc and other possible mechanisms of resistance should be investigated. Also, antiseptic and disinfectant products can vary significantly despite containing similar levels of biocidcs, which underlies the need for close inspection of efficacy claims. In addition, a particular antiscptic or disinfectant product may be better selected (as part of infection control practices) based on particular circumstances or nosocomial outbreaks; for example, ccrtain activc agents arc clearly more efficacious against Gram- positive than Gram-negative bactcria.

4. CONCLUSION

In conclusion, a great deal remains to be learned about the mode of action of antiseptics and disinfectants. Although significant progress has been made with bactcrial investigations, a great understanding of these mechanisms of action will help prevent their microbial resistance. It will also make for more efficient use of these agents clinically with the potential for design of newer, more effective compounds and products.

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AUTHORS' BIOGRAPHY

Abdu Mohammed Alkolaubi has finished ph.d in Egapt 2003 in the feild of microbiology and now working as head department of microbiology in the science collage Taiz University

Jamal Alameri has finished ph.d from Egapt 2009 in the frild of microbiology and working in department of microbiology medical collage Taiz University

Mawhoob Noman Othman Alkadasi has finished ph.d in India 2009 in kuvempu unviresity department in environmental science and working as head department in chemistry zabeed education collage Hudiadah University

Bdulbasit Abdullah Zaid research scholar in mawlana azad BAMu Aurangabad Maharshtra India