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ANTI-BACTERIAL ACTIVITY OF DIFFERENT ANTISEPTICS AVAILABLE IN BENGHAZI-LIBYA HOSPITALS AGAINST PATHOGENIC BACTERIA

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Abstract:-

All over the world, nosocomial infection is a recognized public health problem. They are an essential part of infection control practices and aid in the prevention of nosocomial infections. But a common problem is the selection of antiseptics because different pathogens vary in their response to different antiseptics. Hence the present study was designed to evaluate susceptibility of bacteria isolates from some Benghazi hospitals to five types of antiseptics used in hospitals using the Kirby-Bauer method of disc diffusion method. Data was gathered through a questionnaire. Antiseptics used were Hydrogen Peroxide, povidone- Iodine, Alcoholic Gel, Sterillium (Alcohol 70%), Neo Sterixidina Soap. Bacteria used were Gram positive: Staphylococcus aureus and Streptococcus acidominimus. Gram negative : Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabills and Acinetobacter baumannii, in addition American Type Culture Collection (Staphylococcus aureus ATCC 29213, Escherichia coliATCC 25922 and Pseudomonas aeruginosaATCC 27853) were used as control. The Disc diffusion method technique is a routine, economical and easy screening method used to determine the susceptibility or resistance of a bacterial strain to antibacterial agents. Hydrogen Peroxide 6% and Neo Sterixidina Soap produce the largest zone of inhibition in descending order. The comparative assessment of the zones of inhibition of the diluted antiseptics indicated that Alcoholic Gel, and Sterillium were least effective. While povidone- Iodine 10% the susceptibility was intermediate. This study emphasizes that there was a need to test the quality of antiseptics routinely supplied to the laboratory or hospital to ensure proper control of infections by using right antiseptics in right concentration for a right contact time to reduce the risk of contamination.

Keywords:-*Antiseptics, Pseudomonas aeruginosa, Acinetobacter baumannii*.

INTRODUCTION.

Infection control is an integral part of patient care and hospital setting in order to prevent nosocomial infections. The goal of infection control is to minimize the risk of exposure to potential pathogens and to create a safe working environment in which patients can be treated (1). Health care associated infections (HAIs) are one of the most serious complications of health care. At least one-third of such infections are preventable. Because hands of health care personnel frequently serve as vectors for the transmission of organisms between patients and are also a major reservoir for pathogens with antimicrobial resistance, hand hygiene is one effective strategy to reduce HAIs (2). Two million nosocomial infections happen annually in the United States and it lead to additional days of treatment, increase the risk of fatality and increase management costs (3). Efforts to diminish the risk of transmission of nosocomial infections have a fundamental role (4). Microorganisms invade and colonize surgical wounds, burns, bruises, cuts and open sores in the event of any breach in procedures (5). In 1996 in the United States, 46,500,000 surgical procedures and an even larger number of invasive medical procedures were performed. For example, 5 million gastrointestinal endoscopies are performed per year (6). Ever since the identification of microorganisms as the causative agents of infectious diseases, various methods have been devised in order to reduce the population and prevalence of these organisms. These methods include chemotherapy, immunization, sterilization and disinfection (7). Microorganisms caused hospital infections may be controlled by inhibition or killing by physical or chemical agents as antiseptics, disinfectants, and detergents (8). Antiseptics are agents that destroy or inhibit the growth of micro-organisms in or on living tissue while disinfectant are similar but are used on inanimate objects or surface (9). Subsequently, decontamination, disinfection and sterilization became basic components of any infection control program (10). Biocides are used extensively in hospitals and other health care settings for a variety of purposes. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections (11, 12) and both endemic and epidemic infections and/or diseases (13, 14). The selection, use and control of the effectiveness of disinfectants have been emphasized, since environmental surfaces and medical and surgical instruments can serve as vehicles for infectious agents in susceptible hosts associated with the hospital setting (15). A wide variety of active chemical agents (or biocides) are found, many of which have been used for hundreds of years for antisepsis, disinfection and preservation (13). In general biocides have a broader spectrum of activity than antibiotics, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets (16). But a common problem is the selection of disinfectants and antiseptics because different pathogens vary in their response to different antiseptics or disinfectants (17). The aim of study to evaluate susceptibility of bacteria isolated from some Benghazi hospitals to five types of antiseptics used in hospitals using the Kirby-Bauer method of disc diffusion method.

MATERIALS AND METHODS

Antiseptics used.

Hydrogen Peroxide 6%., povidone- Iodine 7.5%. , Alcoholic Gel, Sterillium (Alcohol 70%) and Neo Sterixidina Soap Bacteria used.

Gram positive.

Staphylococcus aureus. An aerobic Gram positive coccus that produces a smooth to rough colony. Pigment production is varied from gray, gray-white with a yellowish tint, yellowish, or yellow-orange. It may hemolysins. And *Streptococcus vestibularis.*

Gram positive cocci, approximately 1 μg in diameter, that grow in chains.

Gram negative.

Klebsiella pneumoniae: An aerobic Gram negative bacillus that grows readily on most microbiological media producing large mucoid pink colonies when cultured on blood agar and MacConkey agar (Figure 1). *Escherichia coli*. An aerobic Gram negative bacillus that grows readily on most microbiological media. *E. coli* cultivated on blood agar and gave white colonies, on macConkey agar gave lactose fermenting smooth pink colonies. And *Pseudomonas fluorescens*: An aerobic, Gram negative, bacillus that grows well on nonselective media. Some members produce a blue-green or yellow-green fluorescent pigment, others produce a brown-yellow, red-tan, pink, or yellow pigment (Figure 2).

Proteus mirabills: An aerobic Gram negative bacillus that may or may not swarm on most microbiological media.*Acinetobacter baumannii*. An aerobic Gram negative bacillus, gave white colonies on blood agar and pink colonies on MacConkey agar. All Bacteria used identified confirmed by using BD phoenix

Figure 1. *Klebsiella pneumoniae* Culture on MacConkey media

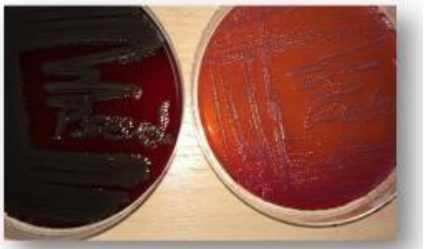


Figure 2. *Pseudomonas aeruginosa* culture on blood and MacConkey media

Indicator Organisms.

In this study freeze-dried bacteria from the American Type Culture Collection (ATCC, USA) were used as control. The organisms used represented both Gram-negative and Gram-positive species. They were *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa ATCC* 27853. These ATCC strains were chosen because they demonstrated an acceptable range of sensitivity to a wide range of antimicrobials and antibiotics (18).

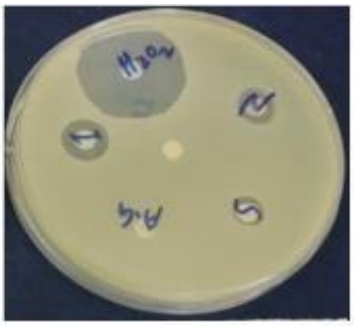
Disc diffusion method.

The agar diffusion technique is a routine, economical and easy screening method used to determine the susceptibility or resistance of a bacterial strain to antibacterial agents. Bacterial was grown on Mueller Hinton agar (MHA-HiMedia, India). It is the commonly used agar to be used in the Kirby Bauer method. For all groups and trials, the petri dishes were all impregnated with the same number of bacteria of 0.5 McFarland which is the standard procedure for the Kirby Bauer method. Standardized filter papers are impregnated in antibacterial for 15 minutes to allow absorption of antibacterial by filter papers. Filter papers are inserted into the Mueller Hinton agar (five in each agar plate), and one in center impregnated in deionized water by the use sterile forceps. The top of the Petri dish is shut. The Petri dishes are put into the incubator set to 35°C for 24 hours. The diameters of the exclusion zones are measured and recorded. The conditions inside the incubator must be the same for all groups and trials to avoid any contribution of factors other than the independent variable (19).

RESULTS

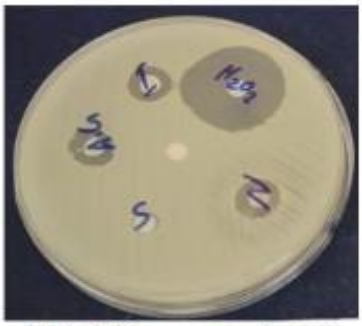
The results show that different types of micro-organisms vary in their response to different types of antiseptic. Hydrogen Peroxide 6% and Neo Sterixidina Soap produce the largest zone of inhibition in descending order. The comparative assessment of the zones of inhibition of the diluted antiseptics indicated that Alcoholic Gel, and Sterilliuwere least effective. While povidone- Iodine 10% the susceptibility was intermediate. Povidone-Iodine 10% showed inhibitory activity on *Acinetobacter baumannii* (S) only, but Hydrogen Peroxide 6% with higher zone of inhibition against all bacteria tested except on *Acinetobacter baumannii* (R). However, Alcoholic Gel, has no effect on these bacteria (Table 1) & (Figure 3, 4, 5 and 6).

This study showed also the antiseptic had an effect on a known strains ATCC obtained from bacterial bank at Benghazi center for infectious diseases and immunology (BCIDI) similar effect on tested bacteria. Hydrogen Peroxide had excellent activity followed by Neo Sterixidina Soap and povidone- Iodine, but Sterillium had low activity while Alcoholic Gel inactive against Indicator Organisms, Table (2) & (Figure 9, 10)



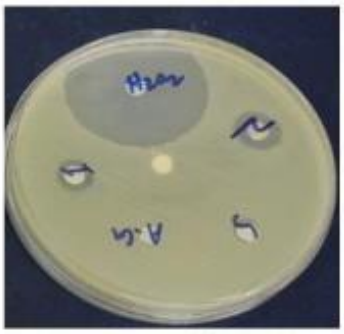
Acinetobacter baumannii (R)

Figure 3. Inhibition zones of Antiseptic against *Acinetobacter baumannii* (R).



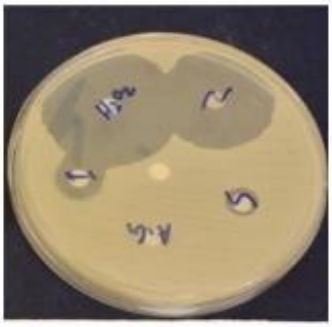
Klebsiella pneumoniae (S)

Figure 4. Inhibition zones of Antiseptic against *Klebsiella pneumoniae* (S).



Proteus mirabills (R)

Figure 5. Inhibition zones of Antiseptic against *Proteus mirabills* (R).



Staphylococcus aureus (R) (MRSA)

Figure 6. Inhibition zones of Antiseptic against *Staphylococcus aureus* (R) (MRSA)..

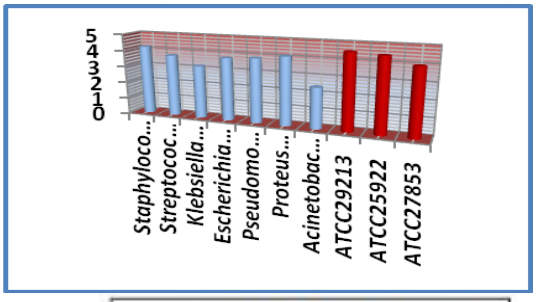


Figure 7. Relation between Inhibition zones of Antiseptic Hydrogen Peroxide 6% and bacteria under study

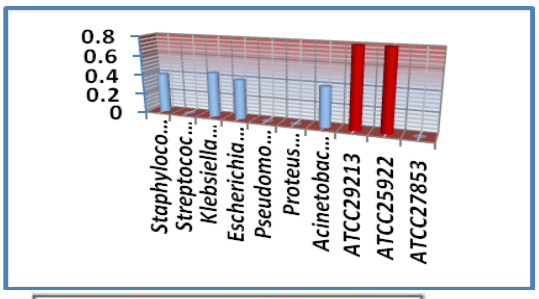
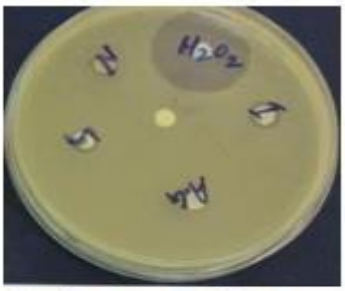


Figure 8. Relation between Inhibition zones of Antiseptic Alcoholic Gel and bacteria under study

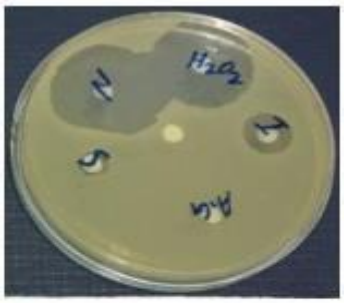
Table (1): The results of Antiseptics by the disk diffusion test.

Antimicrobial / Organism	Zones of inhibition (mm)				
	Alcoholic Gel	Sterillium	Neo Sterixidina Soap	Hydrogen Peroxide	povidone Iodine
Staphylococcus species (S)	0.8	1.1	3.8	4.5	1.5
Staphylococcus species (R)	R	1.0	1.7	3.9	1.1
Streptococcus species (S)	R	1.2	4.5	4.5	2.0
Streptococcus species (R)	R	1.1	4.0	3.0	1.0
Klebsiella species (S)	1.5	0.9	1.4	3.4	1.4
Klebsiella species (R)	0.9	0.8	1.2	3.0	1.2
Escherichia coli (S)	0.8	1.2	3.4	4.5	1.4
Escherichia coli (R)	R	0.8	1.1	3.1	1.2
Pseudomonas species (S)	R	2.0	4.0	4.0	1.4
Pseudomonas species (R)	R	1.0	2.6	3.8	1.2
Proteus species (S)	R	1.2	3.5	4.5	1.2
Proteus species (R)	R	0.8	1.5	3.7	1.0
Acinetobacter species (S)	0.8	1.1	1.3	3.3	3.6
Acinetobacter species (R)	R	1.0	1.0	1.6	1.4



Pseudomonas aeruginosa ATCC 27853

Figure 9. Inhibition zones of Antiseptic against *Pseudomonas aeruginosa ATCC 27853*



Escherichia coli ATCC 25922

Figure 10. Inhibition zones of Antiseptic against *Escherichia coli ATCC 25922*

Table (2): The results of antiseptics against Indicator Organisms by the disk diffusion test.

Antimicrobial / Organism	Zones of inhibition (mm)				
	Alcoholic Gel	Sterillium	Neo Sterixidina Soap	Hydrogen Peroxide	povidone Iodine
Staphylococcus aureus **ATCC 29213**	0.8	1.2	4.0	4.6	1.5
Escherichia coli **ATCC 25922**	0.8	1.2	3.5	4.4	1.5
Pseudomonas aeruginosa **ATCC 27853**	R	2.1	4.0	3.8	1.4

Where: (R) = Resist.

DISCUSSION

All over the world, nosocomial infection is a recognized public health problem, which increasing yearly. Although viruses, fungi, bacteria and parasites are considered as sources of nosocomial infections, bacterial agents remain the most commonly recognized cause (20). Several studies have shown that the frequency of environmental contamination found in the rooms of patients with *Vancomycin resistant enterococci (VRE)* varied between 7 and 37% (21). Even if all colonized inpatients are successfully identified, VRE can spread by healthcare workers through either inadequate hand washing or contact with items like bed rails, sinks and doorknobs (22). The hospital-acquired infection and control such as severe acute respiratory syndrome (SARS) can be prevented and controlled by means of the effective measures such as disinfection and isolation (23).

Management is to prevent infection by inhibiting further microbial invasion of the tissue, thus giving the body a chance to repair itself (24). Infections of wound by microorganism may delay healing, cause failure of healing, and even cause wound deterioration (25). These antiseptics can be used as first aid to reduce bacteria which can compete with host cells for nutrition and oxygen necessary for wound healing (26, 27). The ability of antiseptic to inhibit microorganisms may depend on the mode of action which could be either static or cidal and slow or rapid killer (28). The poor activity of product could be due to an inadequacy of the in use dilution recommended by the manufacturer for utensil disinfection. It is therefore advisable to either reduce the dilution factor or increase the disinfectant surface interaction time (29). Resistance to antibacterial agents can be either a natural property of an organism or acquired by mutation or acquisition of plasmids or transposons (30).

Bacteria are able to adapt rapidly to new environ-mental conditions such as the presence of antimicrobial molecules, and as a consequence, resistance increases with the antimicrobial use (31, 32). So, many hospitals do have such policies test the efficacy of disinfectants to ensure quality. The results show that different types of microorganisms vary in their response to different types of antiseptic. This study showed that antiseptic Hydrogen Peroxide 6% had a great effect with high inhibition zones against bacteria under study by disk diffusion method. Similar study was observed by (17, 33 and 34). In previous studies (17) Iodine showed better antibacterial efficacy not similar the results of the present study were showed the Iodine ineffective by disk diffusion method. However in other previous studies similar the results of the present study were showed that povidone-iodine low effective antiseptic (35). Confirmed the validity of the results by using known strains ATCC, were the antiseptics had the similar effect on tested bacteria and known strains ATCC. All raw data entered into the computer was checked for errors and then analyzed using SPSS statistical software (version 21). Descriptive statistics were used and inferential statistics includes analysis of variance (ANOVA) test and (LSD) for analysis data of disk diffusion method (Figure 7, 8). This study emphasizes that there was a need to test the quality of antiseptics routinely supplied to the laboratory or hospital to ensure proper control of infections by using right antiseptics in right concentration for a right contact time (36). Understanding of the organisms likely contaminating the surfaces and actions of each disinfectant on them. Therefore, it is suggested that the disinfectants and antiseptics which are used in hospitals should be prepared under supervision of hospital health experts (37).

CONCLUSION

In conclusion, the reduced activity of the disinfectants under study may be due to indiscriminate use of these disinfectants in sub-optimal concentrations over a long period of time. The use of sub-optimal concentrations might lead to the development of resistant and virulent strains of organisms. The use of concentrations of disinfectants lower than that quoted by the manufacturers might have serious consequences in the management of patients in hospitals. This study therefore emphasizes the need for hospitals to adhere strictly to standard disinfection policy which gives a guide for proper use of disinfectants and antiseptics. Lack of a universal procedure for surveillance of nosocomial infection, poor hand hygiene, and high level of bacterial contamination on hospital environ-mental surfaces and high prevalence of *Methicillin resistant Staphylococcus aureus (MRSA)* and Pseudomonas as common isolates are the most important problems in our hospitals. In this study was observed Hydrogen Peroxide had a great effect when used for right concentration and the right contact time according to the recommended by the manufacturer. The hospitals must strictly to standard disinfection policy which gives a guide for proper use of antiseptics.

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