Assiut Vet. Med. J. Vol. 61 No. 147 October 2015

A STUDY OF SOME ANTIBIOTICS; DISINFECTANTS AND ANTISEPTICS EFFICACY AGAINST SOME SPECIES OF PATHOGENIC BACTERIA

S.Y.A. AL-DABBAGH; H.H. ALI; I.I. KHALIL and M.A. HAMAD

Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Email: narkikuk@yahoo.com Assiut University web-site: www.aun.edu.eg

ABSTRACT

Received at: 13/10/2015

Accepted: 30/10/2015

İn this study the efficiency of four types of routinely used commercial disinfectant and antiseptics (Ethanol 70%, Dettol –Chloroxylenol- 5%, Hibitine - Chlorohexidine gluconate- 6% and Bleach (Sodium hypochlorite 10%) which used in the laboratory of microbiology in College of veterinary medicine, Mosul, Iraq were tested against four different bacterial strains which isolated from clinical specimens of infected animals (*E.coli, Pseudomonas aeruginosa, Staphylococcus arueus* and *Corynebacterium renale*). Antibiotic sensitivity tests were applied for different nine antibiotics (Ampicilin, Ciprofloxacin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Polymyxin-B, Trimethoprim Sulphamethaxazoll and Penicillin), all the tested bacteria showed resistence for (Ampicilin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Trimethoprim Sulphamethaxazoll and Penicillin). Broth dilution method used for determination of minimum inhibitory concentration (MIC) and disc diffusion method, the results of MIC method after 5 minutes of the exposure to the different concentration of Ethanol, Dettol, Hibitine and Bleach showed that Dettol has no effecincy, followed by Hibitine and Ethanol which showed lower activity while Bleach was the most effective disinfectant on the all tested bacteria. The Gram positive bacteria tested in this study were more sensitive than Gram negative bacteria to all used disinfectant and antiseptics, Disc and diffusion methods had similar effectivness for the tested bacteria against the disinfectants and antiseptics that used in this study.

Key word: *disinfectants, Antiseptics, Pathogenic bacteria, Antibiotic sensitivity test.*

INTRODUCTION

Disinfectants and Antiseptics are widely used as agents for killing or eliminate bacteria especially in microbiological laboratory, hospitals, other humans and animals care centers (MacDonnell and Russell, 1999). The extensively use of disinfectant and antiseptic to control and prevent the growth of microbes in both living tissue and inanimate objects lead to a common problem in the selection of disinfectant and antiseptic against pathogenic microorganisms (Russell and Russell, 1995; Al-Masaudi *et al.*, 1991).

The widespread use of disinfectant and antiseptic products have prompted some speculation on the development of microbial resistance, in particular cross resistance to antibiotics (Russell, 1998). Many chemical agents are now available commercially as disinfectants and antiseptics, these preparations could be halogen compounds, phenols, alcohols, peroxides, quaternary ammonium compounds, chlorohexidine and sodium hypochlorite (Fraise, 1999; Russell *et al*., 1987).

The most commonly used disinfectant in microbiology laboratory are Ethanol, Dettol, Chlorohexidin and soap (Ho-Hyuk Jang *et al.*, 2008), Ethanol, as a dehydrating agent causes cell membrane damage, denaturalization of protein and cell lyses (Larson and Morton 1991). Dettol, effect by denaturation of protein and also act on the cytoplasmic membrane of microorganisms, Bleach with a main constituent of Sodium hypochlorite effect by oxidizing of the cell of microorganism of attaching essential cell component including protein, lipid and DNA, while Hibitin (chlorohexidine) act by disruption of membranes, precipitation of proteins and inactivation of enzymes (Manivannan, 2008).

The antimicrobial properties of the disinfectant agent against some of the pathogenic bacteria have been reported. Moreover, microorganisms are continuously acquiring resistance to new disinfectant and antiseptic (Wisplinghoff *et al.*, 2007). Therefore, it is necessary to evaluate the effectiveness of disinfectant or antiseptic against a specific pathogen so appropriate agent easily selected (Tortora *et al.*, 2013, Brown, 2005).

Antibiotic resistance by various mechanisms has increased worldwide in pathogenic bacteria leading to treatment failures in human and animal infections (WHO, 2007). Bacteria are able to adapt rapidly to new environmental condition include the presence of antimicrobial molecules (Quinn *et al.*, 2004). So that a consequence resistance increases with the antimicrobial uses for pathogenic bacteria (Falagas and Bliziotis, 2007). The successful eradication of these pathogens with antibiotics has been complicated by the development of highly resistant strains as well as the appearance of new virulent pathogens. Some non antibiotic agents to various preparations have been developed and introduced with the aim of breaking the chain of infections in homes, industries and hospitals (Jansen *et al.*, 2006).

MATERIALS and METHODS

Disinfectants and antiseptics:
Four different types of disinfectants and antiseptics as showed in table 1 were used to test susceptibility of the bacterial isolates:

Table 1: Disinfectants and antiseptics used in this study:

Name	Source
Ethanol 70%	Baghdad CO. / Iraq
Dettol (Chloroxylenol) 5%	Ekal industrial CO. Amman/ Jordan
Hibitane (Chlorohexidine gluconate) 6%	Zaid CO. for antiseptic and disinfectant Baghdad / Iraq
Bleach (Sodium hypochlorite) 10%	Sehat. CO./ Iran

Bacterial Strains:
Bacterial strains used in this study were Gram positive (*Staphylococcus arueus* and *Corynebacterium renale*), and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*), all clinical bacterial isolated from infected animals, were properly collected and stored in the Microbiological laboratory, Department of Microbiology, College of Veterinary Medicine, Mosul University, Iraq.

Antibiotic sensitivity test:
All isolates were tested for nine different antibiotics (Bioanalyse) by the standard disc diffusion method according to (Vandepitte *et al.*, 1991) on Muller Hinton agar and incubated for 24 hour at 37℃, those antibiotics included: Ampicilin (Amp)10 µg, Ciprofloxacin (Cip) 5µg, Gentamycin (CN) 10µg, Cefotaxim (Czc)30µg, Cephalothin (KF)30µg, Lincomycin (L) 10µg, Polymyxin-B (pB) 300 U, Trimethoprim sulphamethaxazoll (Tpz) 25µg and Pencillin (P) 10 U.

Sterilization test of used disinfectants and antiseptics:
The four different disinfectants and antiseptics being used in this study were tested for their sterility from microorganisms for accurate sensitivity test as follow, serial dilution (100%, 75%, 50%, 25%). As a negative control one inoculated test tube left without addition of disinfectants and antiseptics while the first dilution (stock) of each disinfectant and antiseptic used in this study considered as positive control. The antimicrobial activity of used disinfectants and antiseptics were tested against 4 types of bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus arueus* and *Corynebacterium renale*) and were isolated from pathogenic animal cases.

Viable Bacterial count:
0.1 ml of each diluted disinfectant and antiseptic were inoculated into plate count agar after 5 minutes of the bacterial inoculation and incubated for 24 hours at 37 ℃.

Minimum Inhibitory Concentration (MIC) method:
The MIC test was determinated according to the method suggested by Baron and Feingo (1990). Depending on the turbidity of the bacterial growth.

Disc diffusion method:
All bacterial strains were cultured on nutrient broth for 24 hr. at 37 ℃, the bacterial inoculums were (5x 10^8 CFU/ ml) according to (Masri *et al.*, 2013). The disc prepared through this study from the same disinfectant and antiseptic used in MIC test and were done according to the method (Wage and Hedin, 1985), the concentration of used disinfectants and antiseptic were (100%,75%, 50%, 25%). for each, Ethanol 70%, Dettol (Chloroxylenol) 5%, Hibitane (chlorohexidine gluconate) 6% and Bleach (Sodium hypochlorite) 10%. The sensitivity test of used disinfectants and antiseptics discs were determined according to (Vandepitte *et al.*, 1991).

RESULTS

Antibiotic sensitivity test were applied for different nine antibiotics, all the tested bacteria showed resistance to (Ampicilin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Trimethoprim Sulphamethaxazoll and Penicillin) but sensitive to (Ciprofloxacin, and Polymyxin-B), as listed in Table 2.

Table 2: Antibiotic sensitivity results for bacterial strains:

Bacterial strains	Amp	Cip	CN	Czc	KF	L	pB	Tpz	P
E.coli	R	S	R	R	R	R	I	R	R
Pseudomonas aeruginosa	R	S	R	R	R	R	I	R	R
Staphylococcus aureus	R	S	S	R	R	I	S	R	S
Corynebacterium renale	R	S	I	R	R	R	S	R	R

R: Resistant, S: Sensitive, I: Intermediate

The results showed that different types of bacteria varied in their response for different types of disinfectants and antiseptics, after 5 minutes of exposure to different concentrations of disinfectants and antiseptics with the comparative of control negative and control positive. Dettol was the least affective against all the tested bacteria in this study followed by the Hibitane (Chlorohexedin 6%) and Ethanol (70%). On the other hand Bleach (Sodium hypochlorite 10%) was the most affected against the tested bacteria (*E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus arueus* and *Corynebacterium renale*), as shown in Figures 1,2,3 and 4.

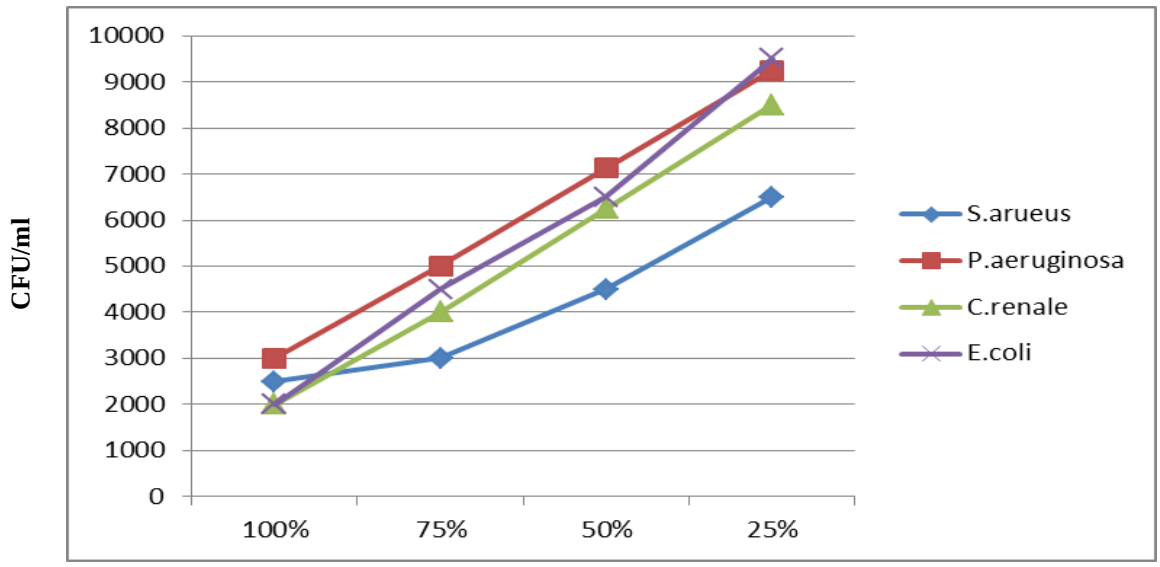

Fig. 1: Viable plate count of different concentrations of Dettol (chloroxylenol) on the tested bacteria.

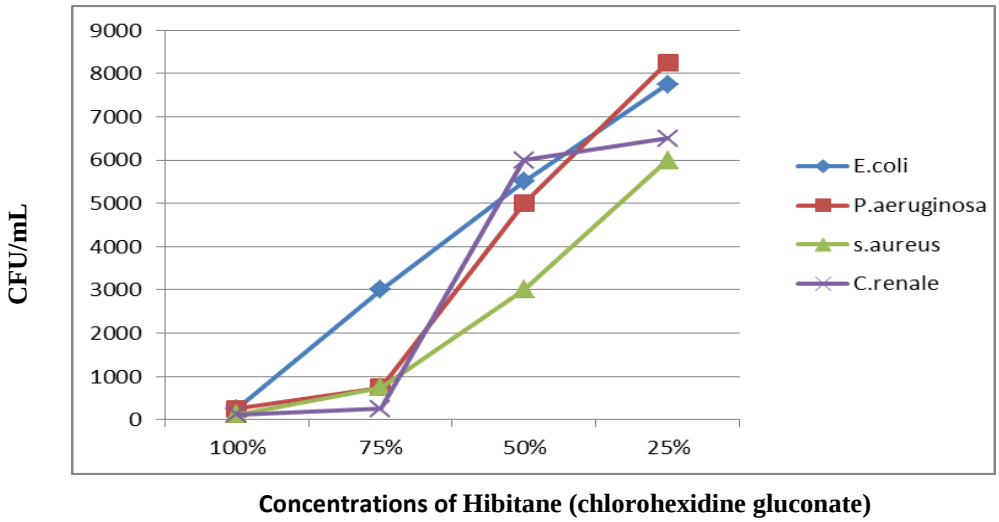

Fig. 2: Viable plate count of different concentrations of Hibitane (chlorohexidine gluconate) on the tested bacteria.

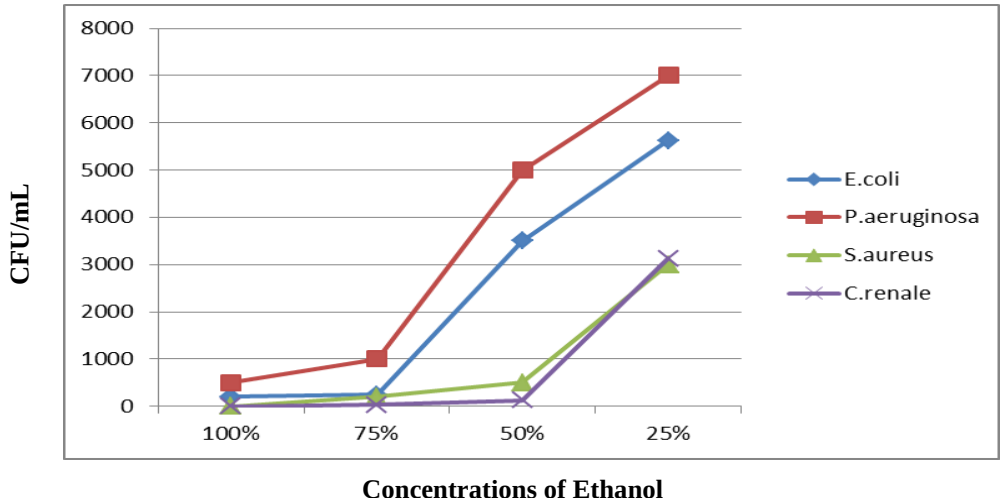

Fig. 3: Viable plate count of different concentrations of Ethanol on the tested bacteria.

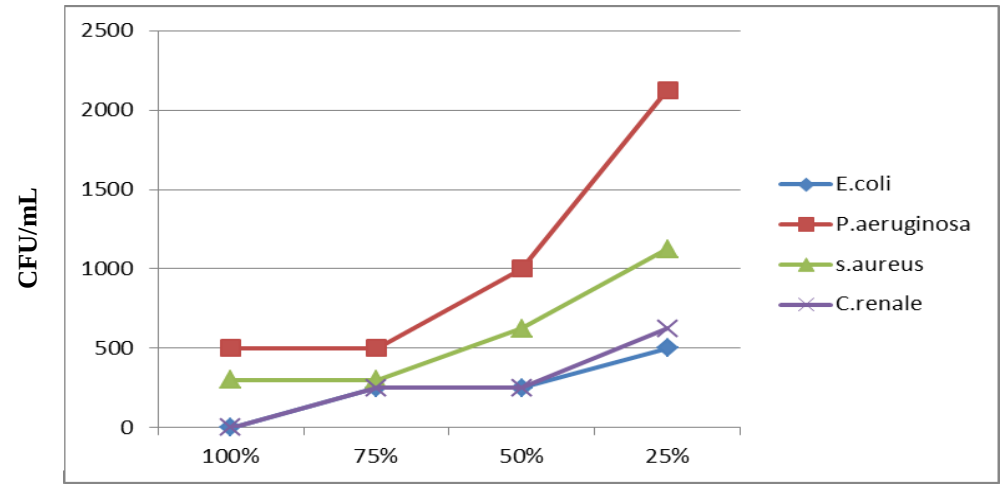

Fig. 4: Viable plate count of different concentrations of Bleach (Sodium hypochlorite) on the tested bacteria.

The zone of inhibition results were differed in their ranges, Dettol was the least effective against all the tested bacteria, the range was varied from 6 to 14 mm for all tested bacteria, non of the four tested bacteria were sensitive to the different concentrations of Dettol, as showen in figure 5.

The different Chlorohexdinie gluconate concentrations showed different effect on the tested bacteria ranged from 8 to 24 mm. *E.coli* was more resistant for most concentrations than the other bacteria ,as shown in figure 6.

The effect of different concentrations of ethanol on the tested bacteria ranged from 6 to 25 mm, *Staphylococcus aureus* was the most sensitive bacteria to all concentrations than the others (figure 7).

Bleach has the best efficiency against the four tested bacteria in all concentrations, the range of the inhibition zones were ranged from 12 to 26 (figure 8).

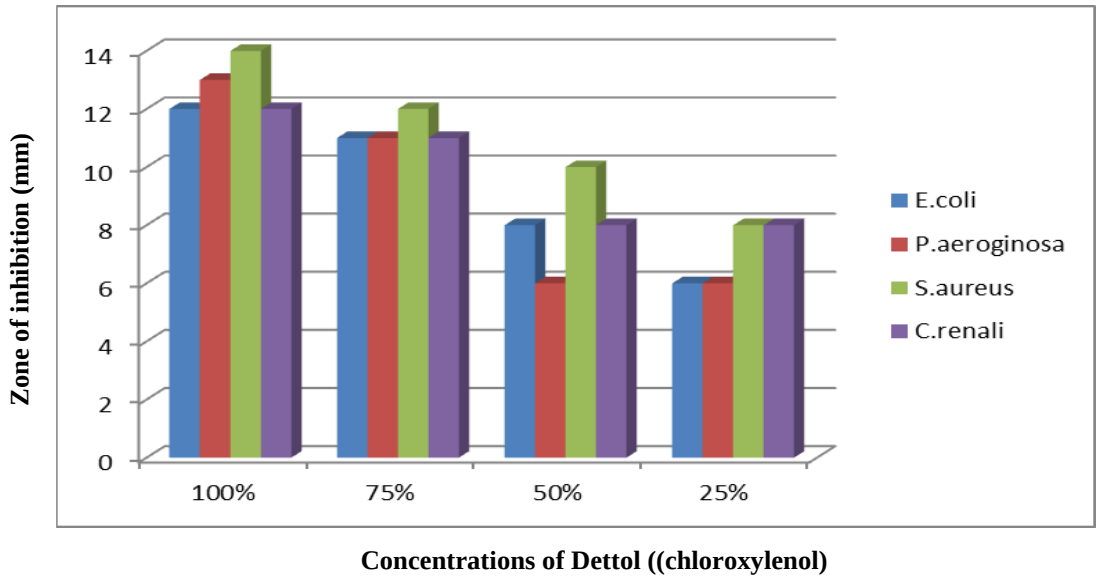

Fig. 5: Inhibition zones of different concentrations of Dettol (chloroxylenol) on the tested bacteria.

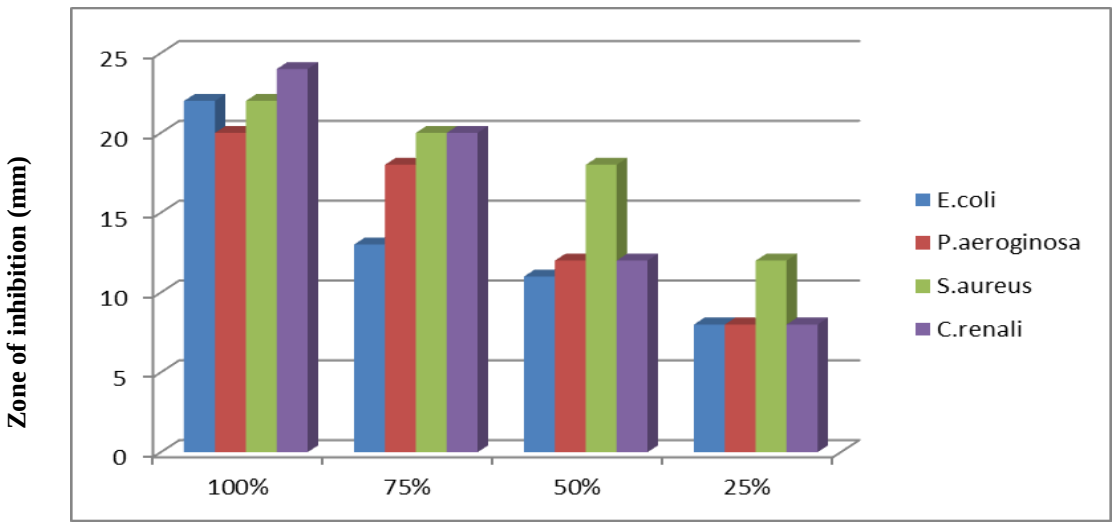

Fig. 6: Inhibition zones of different concentrations of Hibitane (chlorohexidine gluconate) on the tested bacteria.

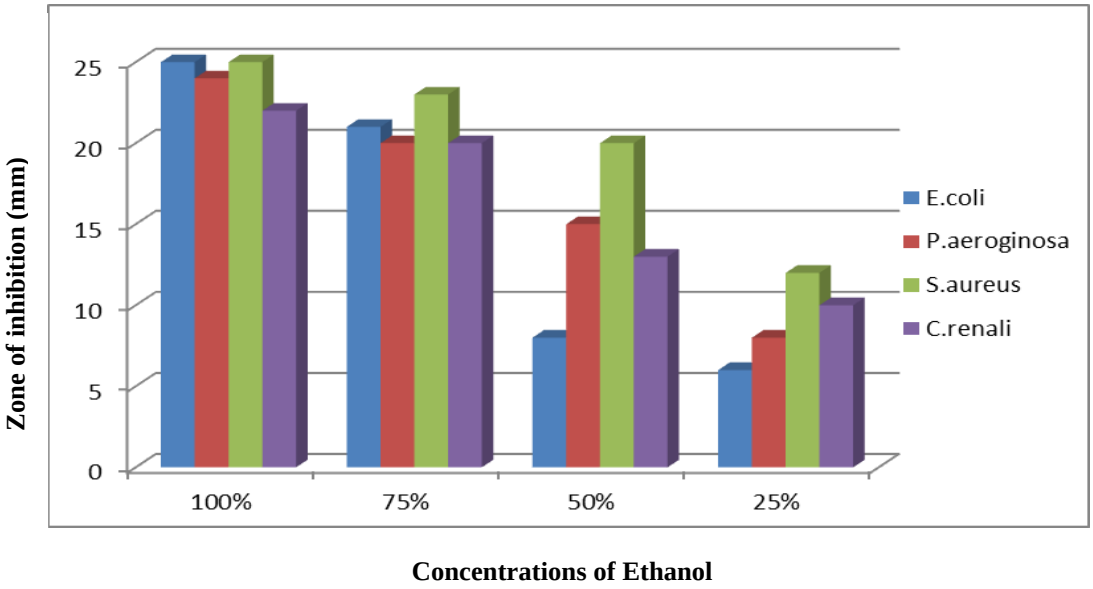

Fig. 7: Inhibition zones of different concentrations of Ethanol on the tested bacteria.

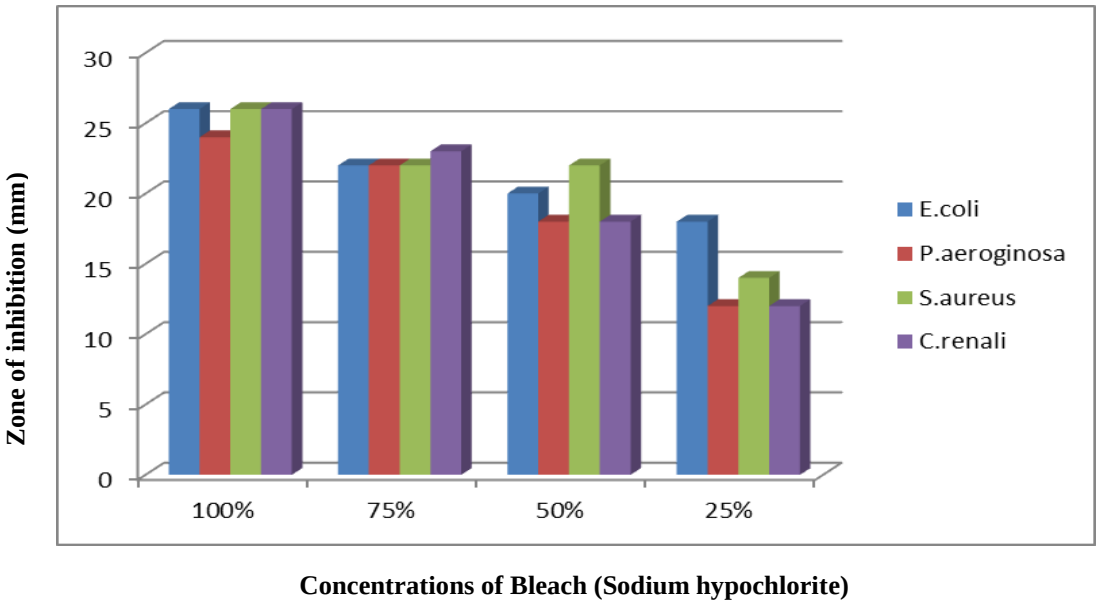

Fig. 8: Inhibition zones of different concentrations of Bleach (Sodium hypochlorite) on the tested bacteria.

DISCUSSION

Disinfectants and antiseptics as antimicrobial products contain approximately 300 different active ingredients, they are marketed in different formation including sprays, liquids, gels, concentrated powders and gases (Mnivannan, 2008 and Bloomfield, 1978).

The extensive use of these disinfectant and antiseptics against the pathogenic bacteria have not only developed resistant but they also grow on the solution of these biocides, all the tested bacteria show resistant to Ampicillin, Cefotaxim, Cephalothin, lincomycin, Trimethoprim sulphamethaxazoll and Penicillin. These results were agreed with (Ayliffe, 1987; El-Mahmood and Doughari, 2009).

In this study the tested *E.coli*, *Pseudomonas. aeruginosa*, *Staphylococcus aureus* and *Corynebacterium renali* isolates showed resistance to Dettol and Hibitin. Ayliffe (1987) reported that bacteria isolated from contaminated disinfectant solutions and antiseptics exhibit increased resistance to commonly used antibiotics that given a fact that bacteria have the ability to share resistant markers and once the resistance develops for one agent, cross-resistance to other agents can occur. Dettol was more effective against *Staphylococcus aureus* than the other tested bacteria, this result agreed with (Saha *et al.*, 2009).

Bleach and ethanol showed high efficiency against the four tested bacteria used in this study, and the obtained results were supported by (Gaonkar *et al.*, 2006). The immediate efficiency of bleach and ethanol was revealed by the high reduction rate in the 30S reaction (Stephen *et al.*, 2004). The immediate killing of bleach can be explained by its oxidizing mechanism, (Fraise, 1999 and Barendra *et al*., 2006) who found similar result, that bleach was rapidly bactericidal for vegetative organisms. The concentration of 10% bleach kill all tested bacteria after 5 minutes of the exposure of this disinfectant. The reason for that results mainly for the mechanism of bleach sterilizing due to oxidation reactions when the bleach is dissolved in water lead to destroy the organisms.

Ethanol was less effective than bleach against the four tested bacteria,as the ethanol sterilization action is mainly due to dehydration of protein and the enzymes to deactivate and prevent bacterial growth (Tortora *et al.*, 2013 and James *et al.*, 1999). The results of inhibition zones were similary to the viable plate count for the effectivness on the tested bacteria that agreed with (Saleh *et al.*, 2012; Masri *et al.*, 2013).

The result showed that Gram negative bacteria were less susceptible to disinfectants and antiseptics. This achieved result agreed with (Saleh *et al.*, 2012) in which the complex cell wall and the outer membrane of these bacteria act as a permeability barrier in limiting or prevention the entry of many chemically types of antibacterial compounds (Russell *et al.*, 1997; Sheldon, 2005).

The whide spread of disinfectent and antiseptic agents have promoted some speculation on the development of microbial resistent (Denyer *et al*., 1985) and this resistance to those agents are mainly of intrinsic nature as the antimicrobial resistant is frequently conferred by plasmid or transposons which have allowed raped and extensive spread through the globe. Development of resistance to antimicrobial agents and biocides is considered as a problem which is compounded by cross-resistance mechanisms between antibiotics and between antibiotics and biocides (Russell, 1986 and Saurina *et al.*, 1997).

As a conclusion, the effecincy of the four disinfectants and antiseptics (Ethanol 70%, Dettol (Chloroxylenol) 5%, Hibitine (Chlorohexidine gluconate) 6% and Bleach (Sodium hypochlorit) 10%) on the four tested bacteria (*E.coli, Pseudomonas aeruginosa, Staphylococcus arueus* and *Corynebacterium renale*) had different efficiency of sterilizing patteren and from the obtained result 10% Bleach had the best efficiency against the tested bacteria followed by Ethanol 70%, while Dettol and Hibitane had less efficiency against the tested bacteria.

REFERENCES

Al-Masaudi, S.B.; Day, M.J. and Russell, A.D. (1991): Antimicrobial resistance and gene transfer in *Staphylococcus aureus*, J. Appl Bacterial 70, 279-290.

Ayliffe, G.A. (1987): Hospital disinfection and antibiotic policies. Chemotherapia 6: 228-233.

Barindra, S.; Debashish, G.; Malay, S. and Joydeep, M. (2006): Purification and characterization of a salt, solvent, detergent and bleach tolerant protease from a new gamma- proteobacterium isolated from the marine environment of the sundarbans, process Biochemistry, 41(1): 208-215.

Baron, J.E. and Feingold, S.M. (1990): Diagnostic Microbiology. 8th ed, Philadelphian: ST Louis: C.V. Mosby company.

Bloomfield, S.F. (1978): The use of disinfectants in the home. J. Appl., 45: 1-38.

Brown, A.E. (2005): Microbiological Application. McGRAW-Hill Companies 35: 217-224.

Denyer, S.P.; Hugo, W.B. and Harding, V.D. (1985): Synergy in Preservative combinations. J. pharm. 25: 245-255.

El-Mahmood, A.M. and Doughari, J.H. (2009): Bacteriological examination of some diluted disinfectants routinelyu used in the specialist hospital yola, Nigeria, African J. of pharmacy and pharmacology, 3(5): 185-190.

Falagas, M.E. and Bliziotis, I.A. (2007): Pandrug-resistant Gram- negative bacteria: the dawn of the post-antibiotic era. Int. J. Antimicrob Agents 29, 630-6.

Fraise, A.P. (1999): Choosing disinfectants. J. of Hospital infection, 43: 255-264.

Gaonkar, T.A.; Geraldo, I.; Shintre, M. and Modak (2006): In vivo efficacy of an alcohol-based surgical hand disinfectant containing a synergistic combination of ethylhexylglycerin and preservatives, J. of hospital infec., 63: 412-417.

Ho-Hyuk, J.; Sung-Ho, A.; Myung- Deok, K. and Chan-Wha, K. (2008): Use of hydrogen peroxide as an effective disinfectant to Actinobacillus ureae.Process Biochemistry, 43: 225-228.

James, R.C.; Daniel, K.N.; Ghassan, S.; Dana, K.J.; Robert, S.D.; Joseph, J.H. and Chung, H.K. (1999): Antimicrobial efficacy of endoscopic disinfection procetures controlled multifactorial investigations, Gastroenistinal Endoscpoy, 50(2): 152-158.

Jansen, W.T.; Van der Bruggen, J.T.; Verhoef, J. and Fluit, AC. (2006): Bacterial resistance: a sensitive issue complexity of the challenge and containment strategy in Europe. Drug Resist Updat. 9, 123-33.

Larson, E.L. and Morton, H.E. (1991): Alcohols. In: Philadelphia, Pa:Lea Febiger, p.191-203.

MacDonnell, G. and Russell, D. (1999): Antiseptics and Disinfectants: Activity, Action and Resistance. Clin. Microbiol. Rev., 12(1): 147-179.

Manivannan, G. (2008): Disinfectant and decontamination, principles, applications and related issues, Taylor and francis Group LLC, London, 87-125.

Masri, N.M.; Hanbali, L.B.; Kamar, A.H.; Kanafani, L.M.S.; Hanbali, M.B. and Haddad, J.J. (2013): The Immunomodulatory, Antimicrobial and Bactericidal Efficacy of Commonly Used Commercial Household Disinfectants, Sterilizers and Antiseptics in Vitro: Putative Anti-Inflammatory Infection Control Mechanisms and Comparative Biochemical Analysis of the Microbial Growth of Gram-Positive Bacteria, American J. of Medical and Biological Research, 1(4): 103-133.

Quinn, P.J.; Carter, M.E.; Markey, B. and Cater, G.R. (2004): Clinical veterinary microbiology. USA: Mosby, an imprint of Elsevier limited, 3-12.

Russell, A.D. and Russell, N.J. (1995): Biocides: activity, action and resistance. Symp. Sog. Gen. Microbiol., 53: 327-365.

Russell, A.D. (1986): Antibacterial action and bacterial resisitance, Infection, 14: 212-215.

Russell, A.D. and Furr, J.R. (1987): Comparative sensitivity of smooth, rough and deep rough strains of Escherichia coli to chlorhexidine, quaternary ammonium compounds and dibromopropamidine isethionate. Int. J. Pharm. 36: 191–197.

Russell, A.D.; Furr, J. and Maillard, J.Y. (1997): Microbial susceptibility and resistance to biocides. ASM News 63: 481-487.

Russell, A.D. (1998): Mechanisms of bacterial resistance to antibiotics and biocides. Prog Med Chem 35, pp 133-197.

Saha, A.K.; Haque, M.F.; Karmaker, S. and Mohanta, M.K. (2009): Antibacterial effects of some antiseptics and disinfectants, J. Life Earth Sci., 3(4): 19-21.

Saleh, R.H.; Naher, H.S. and Al-Jubory, S.A. (2012): A study of efficacy of this disinfectants and bacterial contamination in Al- Hilla Teaching Hospital, Medial J. of Babylon 9(4): 890-899.

Saurina, G.; Landman, D. and Quale, J.M. (1997): Activity of disinfectanr against vancomycin-resistant enterococcus faecium, infect. Control host epideniol., 18:345-347.

Sheldon, A.T. (2005): Antiseptic resistance what do we know and what dose it mean? Clin. Lab.Sci. 18(3): 181-188.

Tortora, G.J.; Funke, B.R. and Case, C.L. (2013): Microbiology an introduction.11 ed. New york: pearson publishing, pp 558-588.clinical bacterioilogy., world health organization, Geneva, Switzerlan.31-36, 78-95.

Wage, S.K. and Hedin, P.A. (1985): Quercetin 3-o-Galactosyl Clucoside a compound fromnarrow leafleted with antimicrobial activity. Phytochemistry., 24: 243-245.

WHO, (2007): A safer future: Global public health security in the 21 century (accessed 25 February 2009).

Wisplinghoff, H.; Schmitt, R.; Wo hrmann, D. and Stefanik, H. (2007): Resistance to disinfectants in epidemiologically defined clinical isolates of Acinetobacter baumannii, Journal of hospital infection 66: 174-181.

دراسة كفاءة بعض المضادات الحيوية، المعقمات والمطهرات على بعض انواع الجراثيم المرضية

سمية ياسين عبد الله الدباغ ، هيفاء حسين علي ، اسراء ابراهيم خليل ، محمد علي حمد

Email: narkikuk@yahoo.com Assiut University web-site: www.aun.edu.eg

تم في هذه الدراسة اختبار كفاءة اربعة انواع من المعقمات والمطهرات التجارية والمستعملة روتينيا (Ethanol 70%, Dettol – Chloroxylenol- 5%, Hibitine -Chlorohexidine gluconate- 6% and Bleach -Sodium (hypochlorite- 10% في مختبر الاحياء المجهرية لكلية الطب البيطري في جامعة الموصل، العراق لفعاليتها ضد اربعة انواع من الجراثيم المعزولة من حالات سريرية لحيوانات مصابة (*E.coli, Pseudomonas aeruginosa, Staphylococcus arueus* and *Corynebacterium renale*) كما تم اجراء اختبارات فحص الحساسية لتسعة مضادات حيوية مختلفة شملت:

Trimethoprin (Ampicilin, Ciprofloxacin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Polymyxin-B (Sulphamethaxazoll and Penicillin

واظهرت النتائج مقاومة الجراثيم المفحوصة للمضادات الحيوية Ampicilin, Gentamycin, Cefotaxim, Cephalothin, Lincomycin, Trimethoprim Sulphamethaxazoll and Penicillin اعتمادا على نتائج اختبارات التخافيف (لتحديد التركيز الادنى المثبط لنمو الجراثيم) واختبار طريقة الانتشار بالاقراص كانت نتائج التركيز الادنى المثبط بعد مرور ٥ دقائق من تعريض الجراثيم لتراكيز مختلفة من الايثانول ،الديتول ،الهبتين والصوديوم هايبوكلورايت. لم يظهر الديتول اي تاثير على الجراثيم المفحوصة بينما اظهر الكحول والهبتين فعالية اقل وكان الصوديوم هايبوكلورايت اكثر المعقمات تاثيرا على الجراثيم التي تم اختبارها في هذه الدراسة. وكانت الجراثيم المفحوصة الموجبة لصبغة الجرام اكثر حساسية من الجراثيم السالبة لصبغة الجرام لمعظم المعقمات والمطهرات ، وقد اظهرت طريقة التخافيف والانتشار بالاقراص نتائج متقاربة في تحديد كفاءة المعقمات والمطهرات التي استخدمت في هذه الدراسة.