In Vitro Evaluation of Antibacterial Activity of Antiseptics and Disinfectants Soled in Babil City.

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ABSTRACT:

In this study (96) swab of clinical pathogenic specimens were collected from Al-Hashimyah General Hospital, by taken from Hospital environment, worker's hands, wards, Hospitalized & admitted patients during 2016.

Specimens cultured directly on enriched and differential media, colonies purified by streaking, then bacteria diagnosed by culture and cell morphology using gram stained slides, finally confirmed by biochemical tests and Api 20 and Api Staph. So, four bacterial species isolated (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa* and *Escherichia coli*).

Antibiotics tested using disk-diffusion method, then use two-fold dilutions method to determine MIC. Primary dilutions of chemical agents were prepared using well-diffusion method on Mueller-Hinton agar, also determined MIC.

Results of bacterial sensitivity tests to antibiotics shows that both Gram +ve and –ve bacteria resist most antibiotics used against them. On bacterial species they resist most antibiotics used except *Strep. pneumoniae* and *E. coli*.

Results of determine time needed to kill multi-drug resistant bacteria after exposure to chemical agents shows that most of these agents were active at (20min), then (15min) and not observed at (5min).

MIC values of different chemical agents affecting on bacteria shows that these results were higher in Bleach and H_2O_2 than others, this indicate potency of them in high concentration to inhibit bacterial growth in culture.

This study conclude the possible of treating bacteria with these chemical agents used, also the emerge of bacterial resistance to both antibiotics and chemical agents, that face its health and public applications.

Keywords: In vitro, antibacterial activity**,** Antiseptics, Disinfectants**.**

التقييم بالزجاج للفعالية ضد البكتيرية المعقمات والمطهرات المباعة بمدينة بابل.

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الخلاصة:

تم بالدراسة الحالية جمع عينات سريرية مرضية من مستشفى الهاشمية العام بأخذ مسحات من بيئة المستشفى وأيدي العاملين بالردهات والمرضى الراقدين والمراجعين خلال عام 2016 بواقع 95 مسحة. زرعت العينات مباشرة على الأوساط الزرعية الإغنائية والتفريقية, إذ نقيت المستعمرات بطريقة التخطيط Streaking, وشخصت البكتريا بدراسة الصفات الشكلية الزرعية للمستعمرات النامية بالطبق وعملت منها شرائح مصبوغة بصبغة كرام للفحص المجهري. وخضعت العزلات للفحوصات البكتيرية المظهرية والكيموحيوية للبكتريا السالبة والموجبة. إضافة لإستخدام عدة تشخيص Api 20 و Api Staph للتشخيص النهائي للبكتريا, إذ تم عزل 4 أنواع من البكتريا المرضية هي: *S. aureus*, *Strep. pneumoniae*, *Ps. aeroginosa* و *E.*

*coli*منها. إستخدمت مجموعة المضادات الحيوية العاملة ضدها بطريقة نشر الأقراص بالطبق وإستخدمت طريقة التخافيف المضاعفة المتسلسلة على الوسط الصلب لحساب قيم التركيز المثبط الأدنى.
حضرت التخافيف الإبتدائية المعقمات والمطهرات الكيمياوية المستخدمة بالدراسة وإستخدمت طريقة الحفر على وسط أكار مولر-هنتون, كما حددت قيم MIC للكيمياويات, بطريقة أنابيب المرق وغيرها.
وأظهرت نتائج فحص الحساسية لعزلات البكتريا تجاه أنواع المضادات الحيوية المستعملة بالدراسة أن البكتريا الموجبة مقاومة لأغلب المضادات المطبقة ضدها بالطبق, وكذلك الأمر بالنسبة للبكتريا السالبة. كما أعطت نتائج فحص حساسية عزلات البكتريا المقاومة للمضادات الحيوية تجاة أنواع العوامل الكيمياوية المستخدمة بالدراسة أن أغلب البكتريا مقاومة للعوامل الكيمياوية المستخدمة ضدها عدا بكتريا *Strep. pneumoniae* و *E. coli*. بينما *S. aureus* و*Ps. aeroginosa* كانت مقاومة لها. أما نتائج تحديد الزمن اللازم لقتل البكتريا المقاومة للمضادات الحيوية بعد تعريضها لأنواع العوامل الكيمياوية المستخدمة بالدراسة فقد بينت أن أغلبية العوامل الكيمياوية المدروسة كانت فعالة عند زمن التعرض 20 دقيقة يليها 15دقيقة ولم تسجل عند زمن تعرض 5دقيقة. ويتضح من قيم MIC للعوامل الكيمياوية المختلفة على البكتريا المقاومة للمضادات الحيوية أن نتائج الـ MIC كانت عالية بكل من القاصر Bleach وبيروكسيد الهيدروجين H_2O_2 وواطئة ببقية العوامل المدروسة, وهذا يثبت قوة هذين العاملين الكيمياويين ضدها بتركيز عالي لتثبيط نموها بمعلق الزرع البكتيري.
إستنتجت الدراسة الحالية وجود إمكانية لمعالجة البكتريا بالدراسة بالمواد الكيمياوية المستخدمة بالتراكيز الطبيعية, وأيضاً ظهور صفة المقاومة البكتيرية تجاه كلاً من المضادات الحيوية والكيمياويات المختلفة والتي تعد مشكلة صعبة تواجه تطبيقها.

الكلمات المفتاحية: الفعالية ضد البكتيرية بالزجاج, المعقمات, المطهرات.

Introduction:

Bacteria compose a higher rate of human environmental contaminants, so increase occurance of nosocomial infections and emerge of resistant strains to more than one antibiotic as its upuse, reuse and missuse, it contains even the bacterial microflora in body, this complicate disease control as not chose the suitable therapy.

The presence of bacterial contamination in house environment be a source of warry, danger and an indicator to cleaning degree and cause diseases, this increased especially after the emerge of antibiotic resistant strains in Hospital and human environment, so researches begans to experiment the use of chemicals and natural active materials as an alternatives against it to prevent its morbidity and mortality.

Disinfectants represent a chemical materials used to disinfect Hospitals, laboratories, healthcare centres and houses, as inhibit and kill bacteria by reduce its numbers and danger, but its upuse, reuse and missuse leads to emerge of resistant strains against it, this contaminate and loss activity of disinfectants.

Sodium hypochlorite (bleach) pH=13.53, from chlorine compounds- Halogens, used to disinfect surfaces floors, tools, equipments, blankets. Have wide microbial range as produce hypochlorous acid a potent oxidizing agent directly unify with proteins and enzymes of cellular membranes to damage cells, bleach efficacy depends on concentration, temperature, pH and time of exposure.

Chlorhexidine (Hibitane) pH=5.87, related to diguanidine compounds, kill bacteria by damage cellular membranes, skin and wounds, also disinfect table surfaces, tools and equipments.

Microbial resistance to disinfectants represent a complicated problem face its use in Hospitals, house and other aspects. Upuse, reuse and missuse produce resistant strains especially when workers unknown its exact concentration needed to kill them, also absence of special staff for disinfection and disefficiency of these disinfectants or be expire or unproperly preserved.

This study aimed to Evaluate of activity (In Vitro) of antiseptics and disinfectants soled in Babil City against common bacterial isolated strains from hospital and house environmennts.

Material and methods:

In present study (95) clinical specimens swabs at Al-Hashimyah general Hospital collected from hospital environment, worker's hands,

wards, hospitalized and admitted patients through 2016.

Bacterial isolation and purification:

Samples were cultured directly directly on enrichment and differential media as blood agar, MacConkey agar, Nutrient agar, Mannitol salt agar (MSA), Eosine-Methylene blue etc. Colonies were purified and picked by streaking method, then plates incubated at 37˚C for 18-24hr, bacterial culture were showed in most plates, bacteria diagnosed macroscopically by study the cultural and morphological properties of growing colonies, then make a gram stained slides for microscopic examination.

Bacterial strains were tested by Gram stain, biochemical tests for G-ve: Indole, Methyl red, Voges-Proscauer, Citrate utilization, Glucose and Lactose fermentation, Catalase, Oxidase, Urease enzymes production. G+ve: Coagulase by slide and tube methods, Catalase, Oxidase, Urease, DNase enzymes production, Mannitol fermentation aerobically, also use of Api20 and ApiStaph (BioMereux, France) for confirmatory test.

Four (4) bacterial species were isolated: *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa* and *Escherichia coli* from various sources, maintained in Brain-heart infusion broth plus 15% glycerol at -20˚C, renewed each three months to serves as stock culture.

Testing bacterial sensitivity to antibiotics:

Carried out using various antibiotic disks listed in table (1), and determine if these strains are sensitive (S), Intermediate (I) or resistant (R) to these antibiotics depends on Kirby-Bauer method in 1966, were antibiotic disks arranged on Mueller- Hinton agar plates aseptically, which contain bacterial culture dilute incubated at 37˚C for 18-24hr, then inhibition zone diameters (mm) on bacterial lawn growth were measured by a transparent metric ruler, read and record results compared with standard tables (NCCLs, 2017).

Two-fold dilutions method were used on agar to determine the minimal inhibitory concentration (MIC) of antibiotics used.

Study effects of various chemical agents on bacteria:

Primary dilutions of antiseptics and disinfectants listed in table (2) were prepared to be a stock solutions from the original commercial concentrations used, to study its effects, well-diffusion method applied on Mueller-Hinton agar by used a cork pork with (5mm), inoculate medium with 0.1 ml bacterial suspension, its turbidity equal the third tube of MacFarland standard (No. 0.5), which contain (1.5×10^8) cell/ml, spreader used to disseminate it on whole plate surface, then add 0.1 ml of each chemical agents by micropipette inside a wells, a control tube contain physiological saline solution only, plates incubated at 37˚C for 24hr, then read and record results by a ruler to measure the inhibition zone diameter (mm).

Also determine the killing time as bacteria that exposed to these chemical agents can't show growth on agar plate. By adds 100µl of bacterial suspension to the agent's tube, then incubated at 37˚C for (5,10,15 and 20min), transferred on nutrient agar plates, incubated at 37˚C for 24hr, then record the results.

Table (1): Antibiotic disks used and standard inhibition zone diameters.

Inhibition zone diameter (mm)			Conc. disc/µg	Code	Antibiotics	ت
9) ≤R(	10-11)I(	12) ≥S(				
13≤	14-16	≥17	10	Am	**Ampicillin**	**1**
17≤	18-20	≥21	10	ME	**Methicillin**	**2**
12≤	13-14	≥15	30	Net	**Netilmicin**	**3**
12≤	13-15	≥16	5	Of	**Ofloxacin**	**4**
9≤	10-11	≥12	30	VA	**Vancomycin**	**5**
19≤	20-22	≥23	30	CZ	**Cephazolin**	**6**
12≤	13-14	≥15	10	GN	**Gentamicin**	**7**
10≤	11-15	≥16	5	TMP	**Trimethoprime**	**8**
12≤	13-17	≥18	30	C	**Chloramphenicol**	**9**
11≤	12-14	≥15	30	Tet	**Tetracycline**	**10**
22≤	23-30	≥31	25	AMX	**Amoxicillin**	**11**
15≤	16-20	≥21	5	CIP	**Ciprofloxacin**	**12**

Table (2): Properties and effects of chemical agents used in study.

Toxic	Irritant		corrosive	Company & source	Concentration	Trade name	Scientific name	Class
	Eye	Skin						
+	+	+		Iraq	5%	Bleach	**Sodium hypochlorite**	**Detergents**
+	+			Jordan	-	Variety	**Soap**	
+	+		+	Samarra	5%	Septol	**Dettol**	**Antiseptics**
+	+			Saudi arabia	6%		**Hydrogen peroxide**	
+	+	+	+	Samarra	7.5%	Betadine	**Povidone-iodine**	**Disinfectants**
+	+			Al-Rahma co., Jordan	4%	Hibitane	**Chlorhexidine**	

Results & Discussions:

In this study 96 swab of clinical pathogenic specimens were collected from Al-Hashimyah General Hospital, by taken from Hospital environment, worker's hands, wards, Hospitalized & admitted patients during 2016. Specimens cultured directly on enriched and differential media, colonies purified by streaking, then bacteria diagnosed by culture and cell morphology using gram stained slides, finally confirmed by biochemical tests and Api 20 and Api Staph. So, four bacterial species isolated (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeroginosa* and *Escherichia coli*).

Antibiotics tested using disk-diffusion method, then use two-fold dilutions method to determine MIC. Primary dilutions of chemical agents were prepared using well-diffusion method on Mueller-Hinton agar, also determined MIC.

Table (3): Results of sensitivity of bacterial isolates to different antibiotics used.

Sensitivity to antibiotics						Bacteria
CZ	VA	OX	N	ME	AM	**Gram +ve**
R	R	R	R	R	R	***S. aureus***
R	S	R	I	R	R	***Strep. pneumoniae***
CIP	AX	Tet	C	TMP	GN	**Gram -ve**
R	I	R	R	R	R	***Ps. aeroginosa***
R	I	I	I	R	R	***E. coli***

Resistant =R Sensitive =S Intermediate =I

Table (3) shows results of sensitivity of bacterial isolates to different antibiotics used, both gram +ve and –ve bacteria gives resistance to most antibiotics used against it on agar plates.

Table (4): Results of susceptibility of antibiotic resistant bacterial isolates to various chemical agents used.

Disinfectants		Antiseptics		Detergents		Bacteria
Chlorhexidine	**Iodine**	**H_2O_2**	**Dittol**	**Soap**	**Bleach**	
I	R	R	I	R	R	***S. aureus***
I	I	R	S	R	I	***Strep. pneumoniae***
R	R	R	R	R	R	***Ps. aeroginosa***
S	I	R	S	R	I	***E. coli***

Resistant =R Sensitive =S Intermediate =I

Table (4) shows results of susceptibility of antibiotic resistant bacterial isolates to various chemical agents used. most bacteria gives resistance to chemical agents except *Strep. pneumoniae* and *E.coli*, while *S.aureus* and *Ps.aeruginosa* resist it.

Recently, bacterial resistance to these chemical agents were generally increased, and especially for G-ve bacilli (weber etal, 2006).

Hospital and house environments were contaminated due to upuse, reuse and misuse of these chemical agents without optimal concentration and time. G-ve mostly more resistant to chemicals than G+ve, as they have external membranes, which decrease their permeability into cell then prevent killing potency. This resistance may be inherited or acquired, and transmit between species leads to emerge resistant atrains (Igbinosa etal, 2015).

Ps.aeruginosa, *Klebsiella* and *Proteus* resist Chlorhexidine and Sodium hypochlorite and gives high MIC values (Riaz etal, 2009).

Synergistic combination of antiseptics were more efficient and suitable for disinfection in recommended concentrations.

These resistant strains be dangerous on health, G-ve more resistant to disinfectants than G+ve (Al-Hadrawi, 2016; Ali, 2015).

Ps.aeruginosa the highest resistant to disinfectants, but sensitive to high concentrations and increase exposure time to disinfectant, this may be related to its cellular structure and inherited resistance to these disinfectants, so differs from others in cause various hard-to-heal infections, which previously be resist antibiotics (Deji-Agboda etal, 2012).

Dettol, soap and detergents were lower resist against it (Ikeghandm etal, 2013).

G-ve *Ps.aeruginosa* and *E.coli more resistant to iodine, than the G+ve as S.aureus* (Assia etal, 2016).

Antibacterial efficacy of chemicals compared with Wolker's Phenol coefficient and test the quantitative suspension who applied on chemical's concentrations, which gives coefficient (5-9), all agents gives fatal effect to bacteria except *Ps.aeruginosa* (Aboh etal, 2013; Rutala etal, 2000).

Full daily cleaning alone be unefficient in decrease bacterial transmission to susceptible patients, but must be supported with targeted and repeated cleaning of surfaces by cloths saturated by an agents (Lei etal, 2017; Massadeh & Jaran, 2009).

G-ve not inhibited by soaps (Butron & Gaikwad, 2009, but against *S.aureus* in wounds and eczema, and must be used moderately to prevent irritation and resistance (Ike, 2016).

In disk-diffusion and turbidity methods *S.aureus were killed rapidly*, as it can't grow in high concentrations, the others gives moderate MIC and high resistance (Bhat etal, 2011), also syudy soap efficacy against it in ditch-plate and hand washing, but the latter not efficient as presence of normal microflora (Mwambete & Lyombe, 2011).

Dettol were high potent disinfectant than others, its bacterial sensitivity decreased with concentration, and its antibacterial efficacy were higher as compared with standard phenol (Chinedu etal, 2015).

Iodine 20% sufficient to kill MSSA (Al-Saadi, 2011), or at used against it in wounds (Talal, 2012).

Sodium hypochlorite related to halogens, its bactericidal effects due to elaborate Hypochlorous acid, a highly oxidizing agent, due to high direct chlorine potential and its compounds unify with cell membrane proteins and enzymes and damage cells (Rutala and Weber, 1997).

High MIC values were determined to H_2O_2, except for *Ps. aeroginosa*, which resist it (Ye etal, 2016; Ahmed & Mashat, 2015).

Chlorhexidine (CHG) related to Biguanides group, used to disinfect skin and membranes, primarily affects on plasma membrane (Al-Nuaimy, 2017; Hameed & Al-Ghanimi, 2016; Al-Mahdawi & Al-Karboli, 2015; Sogawa etal, 2010; Anderson etal, 2010; Penna etal, 2001), G-ve more resistant and its MIC be higher (Ghotaslou & Bahrami, 2012).

Chlorhexidine coefficient, although emerge of bacterial resistance and Sodium hypochlorite inhibit its growth at once and the highest effective at all exposures.

Adds of coefficient substance corelate with sensitivity rates, then decrease resistance (de Silveira etal, 2006).

Table (5) shows the determination of exposure time required to kill antibiotic resistant bacteria after exposure to various chemical agents used in study. Most of these agents efficient at exposure time 20min, then 15min and don't showed at 5min.

Bacterial sensitivity to disinfectants increased with exposure time needed to provide a sufficient time for agent to show its bactericidal effect, bacterial exposure time 15min were unefficient as results of Wijesinghe & Weerasinghe (2010), but some studies said that CHG 4% have antibacterial efficient after 5min of exposure (Ekizoglu etal, 2015).

Table (5) Determined time required to kill antibiotic resistant bacteria after exposed to chemical agents used.

Disinfectants		Antiseptics		Detergents		Time (min.)	Bacteria
Chlorhexidine	Iodine	H_2O_2	Dittol	Soap	Bleach		
						5	*S. aureus*
						10	
+		+	+			15	
	+			+	+	20	
						5	*Strep. pneumoniae*
		+	+			10	
	+					15	
+				+	+	20	
						5	*Ps. aeroginosa*
						10	
+	+	+				15	
			+	+	+	20	
						5	*E. coli*
						10	
+		+	+			15	
	+			+	+	20	

Table (6): MIC values of different chemical agents on antibiotic resistant bacteria (µg/ml).

MIC						Bacteria
Disinfectants		Antiseptics		Detergents		
Chlorhexidine	Iodine	H_2O_2	Dittol	Soap	Bleach	
16	32	2000	64	64	1024	*S. aureus*
32	512	1024	32	32	1500	*Strep. pneumoniae*
128	1024	2000	128	128	1024	*Ps. aeroginosa*
32	32	1500	64	32	2000	*E. coli*

Table (6) shows MIC values of different chemical agents on antibiotic resistant bacterial isolates. MIC results were higher in both bleach and H_2O_2 and lower in other agents, this improve the antibacterial efficacy of these agents in high concentration to inhibit bacterial growth on agar plates.

The study determine MIC values by disk-diffusion and serial dilution methods, then the exposure time of bacteria to disinfectants required to kill these bacterial isolates. Contamination values and reaction of disinfectant and MIC to reduce bacterial numbers to be less than 10^6. Bacterial isolates shows resistance to these chemical agents, this agreed with results of Mazzola etal (2009), also results of Al-Jofi etal (2008) who study Staphylococci resistance to both antibiotics and disinfectants.

References:

Aboh, M.I.; Oladosu,P. & Ibrahim, K. (2013). Antimicrobial activities of some brands of household disinfectants marketed in Abuja municipal area council, Federal Capital territory, Nigeria, Am. J. Res. Comm., 1(8).

Ahmed, O. B. & Mashat, B.H. (2015). Efficacy of three disinfectant agents against contaminating pathogens isolated from public

toilets. Glob. Adv. Res. J. Med. Med. Sci., 4 (112): 473-76.

Akinkunmi, E.O.; Akiyemi, O.O. & olasehinde, O.O. (2015). A study of the antimicrobial effectiveness of diluted antiseptics in Nigeria. British J. Pharmaceut. Res., 8(1): 1-13.

Al-Hadrawi, H.A.N. (2016). Study efficiency and efficacy of some antiseptics used in Al-Sadr teaching hospital in eliminate bacterial contamination. Biol. J Univ. Kufa, 8(1): 9-17.

Ali, Y.H. (2015). Evaluate efficacy of some disinfectants against bacteria isolated from premature's & neoborn's wards in Baghdad teaching hospital, Iraqi J. Nursing Specialists, 28(1): 112-121.

Al-Jofi, I.K.; Al-Hussaini, R.K. & Al-Ubaidy, R.A. (2008). Study resistance of aerobic bacteria isolated from premature's wards to antibiotics and disinfectants. Al-Mustansyriah J. Sci., 19(3): 10-24.

Al-Mahdawi, M.M.A. & Al-Karbuli, M.Y.A. (2015). Study sensitivity of *S.aureus*, *E.coli* and *Ps. aeruginosa* isolated from Al-Ramadi teaching hospital environment to Chlorhextidine and Sodium hypochlorite disinfectants. Al-Anbar J. Vet. Sci., 8(2): 1-6.

Al-Nuaimi, A.A. (2007). Study sensitivity of 3 types of gram –ve bacteria to Chlorhextidine and Sodium hypochlorite disinfectants. Edu. Sci. J., 19(2): 71-81.

Al-Saadi, K.A.H. (2011). Effect of selection pressure of disinfectants on *Staphylococci* resistance type to antibiotics. Karbala Univ. Scientific J. 9(4) Science: 299-304.

Anderson, M.J.; Horn, M.E.; Lin, Y.; Parks, P.J. & Peterson, M.L. (2010). Efficacy of concurrent application of chlorhexidine gluconate & povidone-iodine against six nosocomial pathogens. Am. J. Inf. Control, 38(10): 826-31.

Assia, B.; Leila, D.A.; Rachida, A.; Nedjoua,L.; Nazim, B.; Awatef, B.; Omar, B.; Espigares, M.G.; Moreno, E.R. & Espigares, E.R. (2016). Evaluation of the bactericidal efficacy of different dilutions of tincture of iodine on three bacterial reference strains. J. Chem. Pharm. Res., 8(3): 242-45.

Bhat, R.P.; Prajna,P.S.; Menezez, V.P. & Shetty, P. (2011). Antimicrobial activities of soap & detergents. Adv. Biores., 2(2): 52-62.

Bouzada, M.L.M.; Silva, V.L.; Sa Moreira, F.A.; Silva, G.A. & Diniz, C.G. (2010). Antimicrobial resistance and disinfectants susceptibility of persistent bacteria in a tertiary care hospital, 2(7).

Butrun, W. & Gaikwad, J. (2009). An in vitro analysis of the efficacy of selected bar soaps as antibacterial agents. Bios, 80(2): 66-75.

Chinedu, M.; Stephen, O. & Uchenna, U. (2015). Comparative studies of the efficacy of some disinfectants on human pathogens. Researcher, 7(1): 39-45.

De Silveira, A.B.; Bechtlufft, M.D.; Uander Sand, S.T. & Corcao, G. (2006). Evaluation of the activity of disinfectants against coliform bacteria group strains isolated from a sewage treatment plant (ETE-Ipanema). Acta Sci. Vet., 34(1): 71-76.

Deji-Agboola, A.M.; Onakalu, O.J.; Hassan, A.O.; Adeboyejo, K.S.; Banjo, T.A.; Calebs, B.C.; Adeleke, M.A. & Oluwadun, A. (2012). Efficacy of some liquid antiseptics on *P. aeruginosa* isolated from wounds. Nature & Sci., 10(9): 153-157.

Ekizoglu, M.; Sagiroglu, M.; Kilis, E. & Hascelik, A.G. (2016). An investigation of the bactericidal activity of chlorhexidine digluconate against multidrug-resistant hospital isolates. Turk. J. Med. Sci., 46: 903-9.

Ghotaslou, R. & Bahrami, N. (2012). Antimicrobial activity of Chlorhexidine, Peracetic acid, Hydrogen peroxide & Alcohol-based compound on isolated bacteria in Madani heart Hospital, Tabriz, Azerbaijan, Iran. Adv. Pharmaceut. Bulletin, 2(1): 57-59.

Hameed, N.M. & Al-Ghanimi, N.A. (2016). Evaluation of inhibitory efficacy of hybrid nanochlorhexidine disinfectant against G-ve bacteria in vivo. Karbala Univ. Scientific J. 14(3) Science: 97-105.

Igbinosa, E.O.; Ibhazukur, M.A.; Eribo, O.A. & Ogofure, A.G. (2015). Efficacy of household cleaning agents against some selected pathogenic bacteria, Afr. J. Clin. Exper. Micro., 16(2): 73-8.

Ike, C.C. (2016). Antibacterial activities of different antiseptic soaps sold in Aba on S. aureus from clinical samples. IJRDO J. Biol. Sci., 2(7): 36-47.

Ikegbunam, M.N.; Metuh, R.C.; Anagu, L.O. & Awah, N.S. (2013). Antimicrobial activity of some cleaning products against selected bacteria. Int. Res. J. Pharm. Appl. Sci., 3(4): 133-135.

Jani, S.J.; Mohammed, N.H. & Abdul-Rasoul, M. (2010). Efficacy of various chlorhexidine disinfecting agents on the reduction of bacteria & dimentional stability of alginate impression material, Al-Kufa Univ. J. for Biol Sci., 2(1): 1-10.

Lei, H.; Jones, R.M. & Li, Y. (2017). Exploring surface cleaning strategies in Hospital to prevent contact transmission of MRSA. BMC Inf. Dis., 17:85.

Masaadeh, H. & Jaran, A.S. (2009). Determination of the antibacterial efficacy of common chemical agents in cleaning & disinfection in Hospitals of North Jordan, Am. J. Appl. Sci., 6(5): 811-15.

Mwambet, K.D. & Lyombe, F. (2011). Antimicrobial activity of medicated soaps commonly used by Dar es Salam residents in Tanzania. Indian J. Pharm. Sci., 92-98.

Penna, T.C.V.; Mazzola, P.G. & & Martins, A.M.S. (2001). The efficacy of chemical agent in clearing & disinfection programs. BMC Inf. Dis., 1: 16.

Riaz, S.; Ahmad, A. & Hasnain, S. (2009). Antibacterial activity of soaps against daily encountered bacteria. Afr. J. Biotechnol., 8(8): 1431-36.

Rutala, W.A. & Weber, D.J. (1997). Uses of inorganic hypochlorite (bleach) in health care facilities. Clin. Microbiol. Rev., 10(4): 597-610.

Rutala, W.A.; Barbee, S.L.; Aguiar, N.C.; Sobsey, M.D. & Weber, D.J. (2000). Antimicrobial activity of home disinfectants & natural products against potential human pathogens. Inf. Control Hosp. Epidem., 21:33-38.

Saleh, R.H.; Naher, H.S. & Al-Jubory, S.A. (2012). A study of efficacy of disinfectants and bacterial contamination in Al-Hilla teaching Hospital. Med. J. Babylon, 9(4): 890-900.

Sogawa, Y.; Kobayashi, H.; Kajiura, T. & Nishihava, Y. (2010). Comparision of the residual antimicrobial activity of chlorhexidine- containing antiseptics: An express report. J. Health care associated Inf., 2: 32-36.

Talal, A.K. (2012). Effects of antibiotics and iodine in prevention and treatment of aerobic bacteria cause wound infections in animals. Babylon Univ. J. Pure Appl. Sci., 20(2): 824-836.

Weber, D.J. & Rutala, W.A. (2006). Use of germicides in the home and the health care setting: Is there a relationship between germicide use & antibiotic resistance?. Inf. Control Hosp. Epidem., 27(10).

Wijesinghe, L.P. & Weerasinghe, T. K. (2010). A study on the bacteriocidal efficacy of selected chemical disinfectants & antiseptics. OUSL J., 6(44): 44-58.

Ye, B.M.; Boyko, N.N. & Prysiazhniuk, O.V. (2016). Comparative study of Antimicrobial activity of Peroxydisuccinic acid, Hydrogen peroxide & there mixture, Ann. Mechnikov Inst., 2: 43-49.