Biological activity of 5-benzylidene thiobarbituric acid derivative against some bacteria isolated from burns infections

Mohammed Jasim Al-Shamarti*, Ahmed Hassoon Mageed* and Muslim Idaan Mushin*

الخلاصه

تم جمع 50 عينه من التهابات الحروق من مدينه الصدر الطبيه في محافظه النجف الاشرف خلال الفتره من (2010/1/24 الى 2011/4/4)، اظهرت نتائج التشخيص انواع بكتيريه مختلفه تضمنت *pseudomonas aeurginosa* 33% ، وكانت بكتيريا *pseudomonas fluorescense*16% ، وشكلت كل من *E.coli* و *Klebseilla* spp. 12% و *Aerumonas* spp. شكلت 2% وهي النسبه الاوطئ . اظهرت بكتيريا *pseudomonas aeurginosa* المقاومه الاعلى للمضادات الحياتيه .

تم تحضير 5-benzylidene thiobarbituric acid derivative باستخدام thiobarbituric acid كماده بادئه وتمت معاملتها بـ 4-dimethylaminobenzaldehyde لكي تعطي المركب المطلوب .

تم تشخيص هذا المركب باستخدام احدى طرق spectroscopic method مثل H-NMR, IR CHNS وكذلك بواسطه قياس نقطه الذوبان Melting point. طريقه التحضير البسيطه لـ 5-(4-dimethylaminobenzylidene)thiobarbituric acid تمت باستخدام تفاعل التكثيف condensation reaction لـ4-dimethylaminobenzaldehyde مع thiobarbituric acid في الماء .

توصلت النتائج بأن بكتيريا *Proteus* spp. تتاثر بالمعامله الكيميائيه لهذا المركب اكثر من الانواع البكتيريا الاخرى ، لوحظ من خلال النتائج ان منطقه التثبيط تزداد بزيادة تركيز الماده الكيميائيه. كذلك بكتيريا *Klebseilla* spp، *Pseudomonas.aeurginosa* ايضا اظهرت حساسيه اتجاه هذا المركب في حين بكتيريا *Staphylococcus auerus* لم تظهر اي حساسيه تجاه هذا المركب وعند اي تركيز من التراكيز المستخدمه .

Abstract

A total of fifty (50) samples were collected from burn infections from AL-Sader Medical City in Alnajaf Alashraf during the period from the 24th of January to the 4th of April . Different percentages of different bacteria were noted as follows , *Pseudomonas aeruginosa* was the highest percentage about 33% followed by *Ps. florescence* 8(16 %) , both *E.coli & Klebsiella Sp* comprised 6 (12%) ,and *Aeromonas sp* was the lowest ratio comprising 2% .

*College of Medicine Kufa University

The most bacteria appeared resistance to antibiotics, *Pseudomonas aeruginosa* was found to be resistant to most of the therapeutic agents.

In this paper a synthesis of 5-benzylidene thiobarbituric acid derivative have been described. The route of preparation involved the uses of thiobarbituric acid as starting material and treated with 4-dimethylaminobenzaldehyde compound to give required derivative this compound have been identified by a spectroscopic method like H-NMR, IR and CHNS analysis and also by measuring its melting point.A simple synthetic route for 5-(4-dimethylaminobenzylidene)thiobarbituric acid by the condensation reaction of 4-dimethylaminobenzaldehyde with thiobarbituric acid in water without catalyst is described.

The results revealed that *proteus sp.* was affected by chemical model more than the other bacteria , increasing the concentration of the compound increases the inhibition zone .also bacteria *Klebseilla sp. Pseudomonas aeruginosa*. affected by compound model ,but bacteria *Staphylococcus arueus* was not affected by any concentration of compound.

The aim of of this study was to determine the model of chemical compounds effect on the growth of some bacteria isolated from buruns in vitro.

Introduction

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Data from the National Center for Injury Prevention and Control in the United States show that approximately 2 million fires are reported each year which result in 1.2 million people with burn injuries. Burn patients are ideal hosts for opportunistic infections.(1) The burn site remains relatively sterile during the first 24 hour; thereafter , colonization of the wound by gram negative bacteria is common.(2) *Pseudomonas aeruginosa* has emerged as a predominant member of the burn wound flora and in the absence of topical therapy is cultured from the burn injuries of 70% patients by the third week.[3] Microorganisms routinely isolated from burn wounds include aerobic organisms like *Staphylococcus aureus, Streptococcus pyogenes, E.coli,Klebsiella Spp., Proteus, anaerobic organisms like Bacteroides fragilis,Peptostreptococcus, Propionibacterium Spp., Fusobacterium Spp and fungi like Aspergillus niger, Candida Spp and Zygomycetes* .[4]

The surface of every burn wound is contaminated to some degree by bacteria.[5] Because of this, surface bacterial growth is routinely monitored
in most centers to facilitate management and treatment. It has been found by many
investigators that the distribution of various species of bacteria from burn wound surfaces is similar to that from blood specimens .[6]

Active hydrogen compounds condense with aldehydes and ketones kown as Knoevenagel condensations. These aldol-like condensations usually are catalyzed with weak bases. Iminium ions are intermediates which from α,β-unsaturated compounds having structures corresponding to these formed by mixed aldol condensations followed by dehydration. These reactions are catalyzed by amines or buffer systems containing an amine and an acid are referred to as Knoevenagel condensations.[7] Cross aldol-type condensation of thiobarbituric acid with aromatic aldehydes using acetic acid as a catalyst is available for the preparation.[8] Thiobarbituric acid derivatives are known to possess antibacterial activity,[9]some are claimed to be sedatives,[10] and herbicides,[11] while some are classified as antiviral agents[12] 5-Arylidene thiobarbituric acids are widely used as precursors for the synthesis of bioactive derivatives[13] and its derivatives are also very important intermediates in organic reactions [14] Cross aldol-type condensation of thiobarbituric acid with aromatic aldehydes using acetic acid as a catalyst is available for the preparation.[8] In this paper, we describe a rapid and convenient method for the synthesis of 5-arylidene thiobarbituric acids under uncatalyzed conditions using ethanol as the solvent. It is interesting that the reaction easily occurs in water although the mechanism involves a net dehydration to the alcoholic intermediate obtained by nucleophilic attack of the active methylene neighboring to the carbonyl groups.[15]

Material and methods

Sample collection

Clinical samples were collected from the burns unit of AL_Sader Medical City in Alnajaf Alashraf during the period from the 24th of January to the 4th of April. The swab samples were taken from infected sites of patients.

Isolation and identification

Collected samples were cultured on nutrient , blood and MacConkey's agars for identification . Non –lactose fermented colonies were selected and cultured onto blood agar , then incubated overnight at 37 °C for refreshment and for demonstration of their ability for blood hemolysis .

Identification of samples: The identification of bacteria was performed by the following tests:

Hemolysin production:

A single colony of each isolate was streaked onto blood agar plate and incubated at 37 ºC for 24 – 48 hours. Blood lysis around bacterial growth was recorded .

Urease production:The medium

Triple sugar iron test (TSI-test)

Oxidase test

Catalase test

Motility test

Experimental preparation of chemical compound model :

General

Solvents and materials were obtained from Fluka (Taufkirchen, Germany). Electro thermal 1A melting point apparatus was used to measure the melting point of prepared compound. Infrared spectra were recorded as KBr discs using Fourier Transform Infrared Spectrophotometer FTIR-8400s SHIMADZU. 1H-NMR spectra were recorded by Brukur ,Ultra Shield 300 MHz, Switzerland with TMS as internal standard in DMSO-d6 . Elemental analysis, E. A. G. E. R. -100, Carlo Erba strumentazione, Italy.

Synthesis of thiobarbituric acid

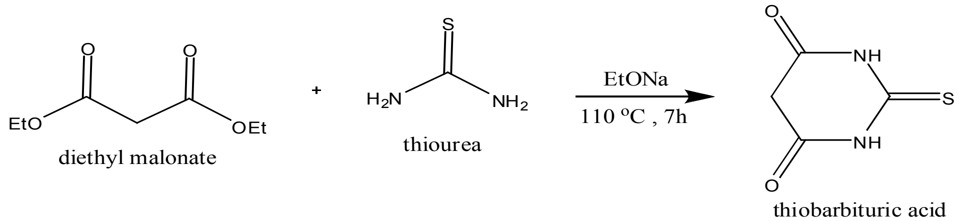
Thiobarbituric acid was prepared according to the literature.(16)

Synthesis of 5-benzylidene thiobarbituric acid derivative

A mixture of 4-dimethylaminobenzaldehyde (10 mmol) and thiobarbituric acid (10 mmol) in water (40 mL) was stirred at 95-100 for 2 hours. Then the solid was filtered and washed subsequently with boiling water, ether. After drying in vacuum .The residue was dissolved in warm ethanol and crystallized (76.92 percent yield) as a red solid , mp 256 oC ; 1H-NMR (DMSO-d6) δ 2.6 (s,6H,N(CH3)2) , δ 6.1 (s,1H, CH) , δ 6.78-7.26 (m, 4H), δ 12.3 (s,1H, NH); FTIR (KBr) 3122 (NH) , 3066(CH) , 1689 (C=O) , 1647 (C=S) , 1606 (C=C) cm-1. Anal. calcd. for C13H13N3O2S: C, 56.71; H, 4.76; N, 15.26; S, 11.65 Found: C, 58.37; H, 5.01; N, 15.57; S, 10.91.

The present investigation involved the application of thiobarbituric acid as starting material which was readily accessible from diethylmalonate and thiourea as shown on (Scheme 1).

Scheme 1. Synthesis of thiobarbituric acid



In this paper, we describe a rapid and convenient method for the synthesis of 5-arylidene thiobarbituric acids under uncatalyzed conditions using water as the solvent (Scheme 2).

Scheme 2. Synthesis of 5-(4-dimethylaminobenzylidene)thiobarbituric acid

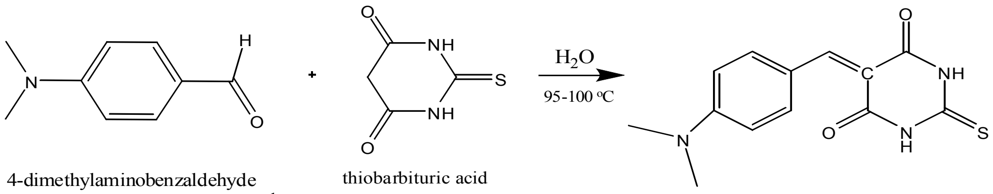
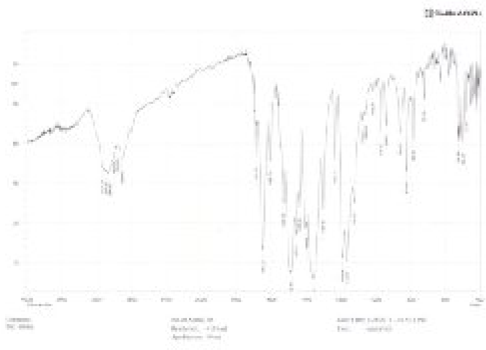
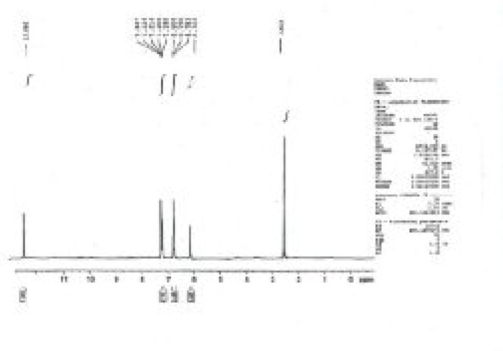


Figure 1. FTIR and ^{1}H-NMR Spectra of 5-(4-dimethylaminobenzylidene) thiobarbituric acid





Antibiotic Susceptibility Test

The test is done by a newly method according to the updates of NCCLs (National Committee for Clinical Laboratory Standards)

Biological Activity Testing of Prepared Chemical Compounds

By using.[17] method for the test of biological activity of the prepared chemical compounds which includes the following steps:

1. Prepare bacterial suspension and compare with McFarland tube 0.5 .
2. Spread bacterial suspension on (Muller Hinton Agar) homogeneously (0.1 ml) to cover the whole surface. Make holes in the agar by using 6 mm diameter cork piercing.
3. Prepare diluted solutions (5,10,15,20,)mg/ml for each compound at physiological pH(7).
4. Put the prepared solutions in holes to investigate their biological activity .
5. Incubate the petri-dishes at 37C. for 24 hours.
6. Measure the diameter of inhibition zone for each hole by the ruler to determine the effectiveness of each compound and compare with the standard limits of sensitivity of the same species of bacteria against antibiotics .

Statistical Analysis

The analysis of the results was according to the model testing process and the design was higher of accuracy and tested incorporeal results by using a least significant difference(L.S.D.) below the level of probability P≥0.05 based.[18]

Result and discussion

In this study number of isolated *Pseudomonas aeruginosa* 25 (33%) , *Pseudomonas florescence* 8 (16%*) E.coli* & *Klebsiella. sp* 6 (12%) while *Proteu*s 4(8) and Aeromonas sp 2(3%) these result different with a study conducted[19,20] *Ps.aeruginosa* isolated from (65%). In their study have demonstrated that infections by gram positive organisms were more common in first 5 days of burns while gram negative organisms dominate the infection scene thereafter.[21] Either[22]reported increase in percentage Ps. aeruginosa &S.areus if percentage 35% ,23% consecutively. In Study of the Turky reported[23] the high number isolated from burns *ps. aeruginosa* 55% *S. aureus* 40%.while report [24] ps. *aeruginosa* 20% ps. *florescence 25%* . (39.5%) , [25] isolates from burns infections(39.5%) isolates from *Klebsiella . sp.*

The results shown in Table(1) revealed that 50 samples(69%) gave positive bacterial culture whereas 25 (30.8%) showed no bacterial growth. Regarding skin swabs, were positive bacterial cultures consisting of single growth 35(83.6%), and mixed bacterial growth 9(16.4%). Meanwhile, no bacterial growth was found in 6 (14.1%) of skin swab cultures. The single and mixed bacterial growth results of skin swabs are shown in Table(1). These results agree with that obtained [26] who found that 86.5% of skin swabs were positive for bacterial growth. Also[27] reported that negative bacterial growth was found in approximately 18% of the cultures of skin swab.

Table(1) Numbers and Percentages of Bacterial Isolates from Burn Patients and Burn Unit

Result	Skin No. (%)	Burn Unit No. (%)	Total of Samples No. (%)
Culture Positive	33(85.9%)	17(56.7%)	50 (69.2%)
Single Growth	15	10	35(83.3%)
Mixed Growth	9	6	15 (16.7%)
Culture Negative	12(14.1%)	13(43.3%)	25 (30.8%)

Table(3) Number and Percentages of Bacterial Isolates

Aeromonas.sp	Enterobacter	E.coli	Proteus.mirabilis	Klebsilla.sp	S. aureus	Ps. Florescence	Ps. aeruginosa	No.
2	3	5	4	6	6	8	25	NO. Isolated
3%	6%	10%	8%	12%	12%	16%	33%	Total Percentage

Table (2) Biochemical Tests

Test / sample	Results														
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S`10	S11	S12	S13	S14	S15
Oxidase	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Gelatin Liquefication	+	+	+	-	+	+	-	_	+	+	+	+	+	+	+
Gram-stain	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Urease	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-
Hemolysis(ß-hemolysis)	+	-	+	+	+	+	-	-	+	+	+	+	+	-	-
H2S production	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-
Indole test	-	-	-	+	-	-	+	+	+	+	-	-	-	-	-
Kligler's iron agar	K/A	K/A+Gas	N.C	A/A	K/A	K/A+	K/A+	K/K	A/A	A/A	N.C	N.C	N.C	A/A+	K/A+Gas
Protease test	+	+	+	+	-	+	+	-	_	+	+	+	+	+	+
Methyl-red	-	-	-	+	-	-	-	-	+	+	_	-	-	+	-
Motility	+	-	-	+	-	+	+	-	+	+	-	-	-	+	-
Fermented lactose	-	+	-	+	+	+	-	-	+	+	-	-	-	-	+
Pigments production	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-
Simmon's citrate	+	+	-	_	+	+	+	-	-	-	+	+	+	-	+
Voges-Proskauer	-	+	-	-	+	+	+	+	-	-	-	-	-	-	+

+ : positive test, - : negative test, K : Alkaline

3: Antibiotic Susceptibility

The susceptibility of isolates for common antibiotics was studied to determine the pattern of isolates resistance to various antibiotics depending on disk diffusion method. The antibiotics represented by, Trimethoprim , tetracyclines, Ciprofloxacin, and others.

The results showed that *Ps. aeruginosa* was the most resistant bacteria to all antibiotics also *Klebseilla.sp, and staphlycoccus aureus* but some isolates were sensitivity to Ciprofloxacin and Gentamicin , this result goes in agreement with the result[24,25] ; the results recorded that *proteus* , *Aeromonas* , *Enterobacter* and *E. coli* were less resistant than *Ps. aeruginosa, Klebseilla.sp,* these isolates were sensitive to Ampicillin,: Tetracyclin, Cefotaxime[28] ,this resistance is related to the genes carried on plasmid or chromosome [29-31] ,produce several extended spectrum β-laclamase enzymes which inhibit the action of β-lactam antibiotics, conferring bacteria the ability to persist under β-lactams treatment, as recorded.[25]

Table (4) The resistance of Bacteria isolates to antibiotics

Antibiotic disc											NO.
Am	Cl	Te	CTX	Cip	KF	TMP	N	Ax	CN	S	
R	R	R	R	R	R	R	R	R	R	R	*Ps. aeruginosa*
R	R	R	R	S	R	R	R	R	R	R	*Ps. Florescence*
R	R	R	R	R	R	R	R	R	R	R	*S. aureus*
R	R	R	R	R	R	R	R	R	R	R	*Klebseilla.sp*
R	R	S	R	S	R	R	R	R	R	R	*E.coli*
R	R	R	R	R	R	R	R	R	R	R	*Proteus .sp*
R	S	R	S	R	R	S	S	R	S	S	*Enterobacter.sp*
R	R	R	R	R	R	R	R	R	R	R	*E.coli*
R	R	R	R	R	R	R	R	R	R	R	*Klebseilla.sp*
R	R	R	R	R	R	R	R	R	R	R	*Ps. aeruginosa*
R	R	R	R	R	R	R	R	R	R	R	*Ps. aeruginosa*
R	R	R	R	S	R	R	R	R	R	R	*Ps. aeruginosa*
R	S	S	R	S	R	R	R	S	R	R	*Aeromonas sp*
R	R	R	R	R	R	R	R	R	R	R	*Klebseilla.sp*

Am: Ampicillin, Te: Tetracyclin, CTX: Cefotaxime, Cip: Ciprofloxacin, CN: Gentamicin, Ax: Amoxicillin, Cl: Cephalexin, , N: Neomycin, KF: Cephalothin, Trimethoprim

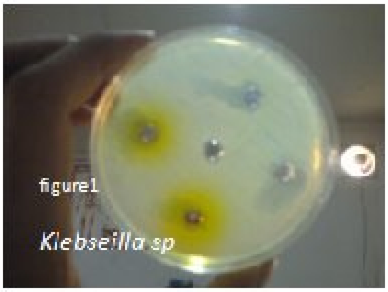
4: Study of Biological Activity :

The results showed that *Ps. aeruginosa*, *Klebseilla .sp* and *Proteus .sp* , were sensitive to compound 5-benzylidene thiobarbituric acid derivative, and increasing the compound concentration increases the inhibition zone. but Staphylococcus arueus was found resistant in vitro. this different back to biological activity of this compound ,it has effecting groups working on bacteria metabolism, the compound model was capable to combine with chemical material in cell after that become complex inside the cell, the bacterial will become dehydrated and die .this result applicator with [25] used the prepare compound 2-[4-(dimethylamino)-3-[(4- methoxyphenyl) diazenyl]phenyl]-3-(aryl)-2-hydrobenzo[e]-1,3-oxazepine-4,7-dione) and also used prepare DBHBED found was effect to E.coli this compound effect to my be on metabilsim of this bacteria [25] .This might be due to the change in the genetic properties of the living cells.[32]

Table (5) biological activity to model compound on some genus of bacteria

Inhibition zone				Con. mg/ml
Ps. aeruginosa	*S. aureus*	*Proteus .sp*	*Klebseilla.sp*	
0	0	0	0	5mg /ml
0	0	0	0	10 mg/ml
20	0	30	25	15 mg/ml
22	0	33	30	20mg /ml
1.2	0	1.2	3.5	LSD

.



References

1. Cochran A, Morris SE, Edelman LS, Saffle JR. 2002. Systemic Candida infection in burn patients. Surg infection Larch mt. Vol.3(4).pp367-374.
2. Pruitt Jr BA, McManus AT, Kim SH, Goodwin CW. 1998. Burn wound infections: Current status world Journal of Surgery. Vol.22.pp135-145.
3. Church et.al. 2006. Burn wound infections. Clinical Microbiology Reviews. Vol.19(2).pp 403 - 434.
4. Revathi G, Puri J, Jain B K. 1998. Bacteriology of burns. Burns. Vol.24.pp347-349
5. Lawrence JC, Lilly HA. 1972. A quantitative method for investigating the bacteriology skin: its application to burns. Journal of Exp Pathol. Vol.50.pp550-559
6. Li GH. Chung Hua Cheng Hsing Shao Wai Ko Tsa Chih. 1989. Analysis of microbiological flora in the blood and wounds of burn patients. Vol.5.pp199-200,pp 238-239.
7. P. Schuster, O. E. Polansky, and F. Wessely, *Monatsh*. 1964, 95,53.

8. I. D.yachkov, B. A. Ivin, N. A. Smorygo, E. G. Sochilin, *Zh. Org. Khim*., 1976, 12, 1115.
9. L. K. Akopyan, A. S. Adzhibekyan, G. A. Porkinyan, E. A. Tumasyan, *Bilzh. Arm*. 1976, *29*, 80; *Chem. Abstr*. 1976, *85*, 72068.
10. S. Senda, H. Fugimura, H. Izumi, *Japan Patent* 193,6824, 1968; *Chem. Abstr*. 1969, *70*, 78001.
11. S. L. Katz, A. W. Gay, *U.S. Patent* 352,806, 1982; *Chem. Abstr.,*1983, *98*, 215603.
12. Esanu, *BE Patent* 902,232, 1985; *Chem. Abstr*. 1986, *104*, 130223.
13. B .Reijo, H. Erkki, *WO 9117151*, 1992, *116*, 59398 .
14. H. Ikeda, Y. Kawabe, T. Sakai, K. Kawasaki, *Chem. Lett*. 1989, 1803.
15. Q. YANG, J. LU, M. TIAN, *Chinese Chemical Letters* ,2003 ,14, 1240.
16. N. T. Fan, *Examples of Organic Synthesis*, Beijing University of Science and Technology Press, Beijing, 1995, p. 215.
17. N. S. Ego rove. , Antibiotics Scientific Approach . Mir publishers. Moscow., 1985.
18. W. Daniel ,Biostatistics: A foundation for Analysis in the health sciences .2nd Ed. John Wiley and sons, 1978.
19. El-Sonbaty, M.A. and El-Oteify, M.(1990). Epidemiology of burns in Assiut province during the last two years. Assiut medical journal.
20. Attia, A.F.; Sherif, A.A.; Mandil, A.M.; Massound, M.N; Abou-Nazel, M.W. and Arafa, M.A.(1997). Epidemiological and sociocultural study of burn patients in Alexandria, Egypt. Eastern Mediterranean Health Journal. 3:452-61.
21. Ahmad, M.; Shahid, H.S.; Ibrahim, K.M. and Malik, S.A.(2006). Pattern of bacterial invasion in burn patients at the Pakistan institutes of medical sciences, Islamabactt. Annals of burns and fire Disasters. 19 (1).
22. Zhang, Y. H. ; Deng, S. L. and Liu, J. W. (2005). Analysis of susceptibility of Pseudomonas aeruginosa isolated from burns toward antibiotics in vitro Zhonghue. Shao. Shang. Za. Zhi. 21 (2) : 104 – 106.

23. Yildirim, S. ; Narsal, T. Z. ; Tarim, A. ; Torer, N. ; Noyan, T. ; Demiroglu, Y. Z. ; Moray, G. and Heberal, M. (2005). Bacteriological profile and antibiotic resistance : comparison of findings in a burn intensive care unit , other intensive care unit , and the hospital services unit of a single center, J. Burn. Car. Rehabil. 26 (6) : 488 – 492.
24. Muhsin ,I. Muslim.(2010). The Effect of Phosphate on Growth and Virulence Pseudomonas aeruginosa In vitro and In vivo. M.Sc. Thesis College of Science, Kufa University. Iraq.
25. Al-shamarti ,J. Mohammed. (2010). Molecular Evaluation of β-lactam Resistance Genes in Klebsiella spp. Isolated from Clinical Cases in Al-Najaf Province . M.Sc. Thesis College of Science, University of Kufa. Iraq.
26. Bagdonas, R.; Tamelis, A.; Rimdeika, R. and Kiudelis, M.(2004). Analysis of burn patients and the isolated pathogens. Lithuanian Surgery. 2(3):190-3.
27. Al-Akayleh, A.T. (1999). Invasive burn wound infection. Annals of burns and fire disaster. 12(2).
28. Hujer, A. M., K. M. Hujer and R. A. Bonomo (2001). Mutagenesis of amino acid residues in the SHV-1□-lactamase: the premier role of Gly238Ser in penicillin and cephalosporin resistance. Biochim Biophys Acta 1547: 37-50.
29. Aubron, C.; Poire, L.; Fortine, N.; Nicolas, p.; Collet, L. and Nordman, P. (2005). Nosocomial spread of Pseudomonas aeruginosa isolates expressing the metallo-beta – lactamase UIM-2 in a hematology unit of a French Hospital. Microb. Drug. Resist. 11 (3) : 254 – 263.
30. Martinez, J. L. and Baquero, F. (2002). Interactions among strategies associated with bacterial infections : Pathogenicity , epidemicity and antibiotic resistance. C. M. R. 15 (4) : 647 – 679.
31. Pagani, L. ; Colinon, C. ; Migliavacca, R. ; Labonia, M. ; Docquier, J. D. ; Nucleo, E. ; Spalla, M. ; LiBergoli, M. and Rossolini, G. M. (2005). Nosocomial outbreak caused by multi drug resistance Pseudomonas aeruginosa producing IMP – 13 Metallo – beta – lactamase. J. Clin. Microbiol. 43 (8) : 3824 – 3832.
32. J. C. Jung ; J. C. Kim; O. S. Park; B. S. Jang, Arch. Pharm. Res. , 22, 302, 1999.