(Comparison of some antibiotic effect with the effect of Laboratory prepared compound)

Nisreen Abdul karirm Abdul Aali
Department of Chemistry –College of Science
Misan University.

Abstract:

Pharynx Swabs were taken from **40** patients with Pharyngitis during the period (**25 / 4 to 5/25/2017**). Samples were cultured , the colonies diagnosed by the Biochemical tests. ***Streptococcus pyogenes*** isolated in ratio of 30%, the antibiotic susceptibility tests were performed on ***S. pyogenes*** and other clinical isolates. All isolates were more susceptible to Ciprofloxacin, Amoxicillin and Azithromycin successively. The susceptibility of the Isolates to Ciprofloxacin were approximately similar to it's' susceptibility when using the laboratory prepared compound A(2-furancarboxylic acid,tetrahydro-5-oxo-ɣ-Butyra lactone- ɣ-carboxylic acid) the structure of the synthesized compound was confirmed by IR spectra.

الخلاصة :

اخذت مسحات من البلعوم من ٤٠ مريض مصاب بالتهاب البلعوم للفترة من(٤/٢٥ الى ٥/٢٥ /٢٠١٧) زرعت العينات وشخصت العزلات باستخدام الاختبارات الكيمو حيوية ظهرت***S.pyogenes*** بنسبة عزل ٣٠% اختبرت الحساسية الدوائية للـ ***S.pyogenes*** وباقي العزلات السريرية وكانت العزلات الظاهرة حساسة اكثر للـ ciprofloxacin يليه Amoxicillin ثم Azithromycin كما أظهرت العزلات حساسية مقاربة لحساسيتها تجاه الـ ciprofloxacin عند استخدام لمركب المحضر مختبريا
(2-furancarboxylic acid,tetrahydro-5-oxo-ɣ-Butyra lactone- ɣ-carboxylic acid).

Introduction

Streptococcus pyogenes is a gram positive bacterium, with spherical or ovoid shape, the cells are arranged in chains, grows on many culture media enriched with 5% of human or sheep blood as discoid colonies usually 1-2 mm in diameter generally produces large zones (1cm) of beta hemolysis when cultured on blood agar plates, and are therefore also called Group A [1,2].

S.pyogenes can causes important human diseases including tonsillitis, pharyngitis, and skin infections in addition to pos-infection complications such as rheumatic fever and glumerulonephritis [3].

The percentage of isolation of *S. pyogenes* in pharyngitis and tonsillitis ranging between (10-22%) comparing with other species which appear in bacterial culture [4].

S. pyogenes is susceptible penicillin and its derivatives [5,6] It is also very sensitive to the treatment by Amoxicillin [7] a complete eradication of *S. pyogenes*

after the using a regular dose (775 mg for a 10-day regimen)of Amoxicillin in adolescents and adults. The bacterium is susceptible to ciprofloxolan and Azithromycin too [8].

In recent years, *S. pyogenes* showed a ratio of resistance to various antibiotics such as Erthromycin and Clindamycin[9,10,11].The emergence of resistance to antibiotics in different types of bacteria have encouraged a tendency to use compounds produced naturally or synthetically working as anti-bacterial agents and alternate the commercial use of antibiotics. Some polymers have an antibacterial properties by having some active groups effective against proteins, enzymes and DNA of the bacteria [12].

The aim of the present study is to evaluate the effectiveness of laboratory prepared compound(2-furancarboxylic acid,tetrahydro-5-oxo-ɣ-Butyra lactone- ɣ-carboxylic acid) on the bacteria and comparing it with antibiotics sales in the markets.

Materials and methods

Sampling

Throat swab samples were collected using a sterile wooden swabs during the period of 25/4 till 25/5/2017, 40 samples in ages ranging from (7-30) years for both genders. All samples were cultured on blood agar immediately after sampling.

Preparation of culture media

Blood agar was prepared by dissolving 28 g of blood agar base in 1 liter of distilled water, after sterilization by autoclave the media left to cool till 45^{o}c in the room temperature, a proportion of (5-10%) of blood were added, the media poured in Petri dishes and left to be solid then the samples were cultured by streaking technique immediately and incubated in 37^{o}c for 24 hrs.

Biochemical tests

All grown isolates in primary culture were activated singly by growing them on new blood agar plates in 37^{o}c for 24 hrs, that was prepared for performing a following biochemical test:

1. Gram stain
2. Catalase test: was performed by putting a part of grown colonies on glass slide and adding drops of (0.3% H_2O_2). The production of gas bubble indicating the positive result.
3. Oxidase test: was performed by putting a part of grown colonies on a filter paper flooded with oxidase enzyme solution. The paper color changing form colorless to violet indicating the positive result.
4. DNase test: the medium was prepared by dissolving (DNas agar 42g), (manittol 10g) and (Bromothymol blue 0.025) in 1 liter, the mixture was

sterile by autoclave and poured in Petri dishes, after solidity a loopful was laken from activated isolates, cultured and incubated in 37°c for 24 hrs.

5. susceptibility to Bacitracin and Optochin

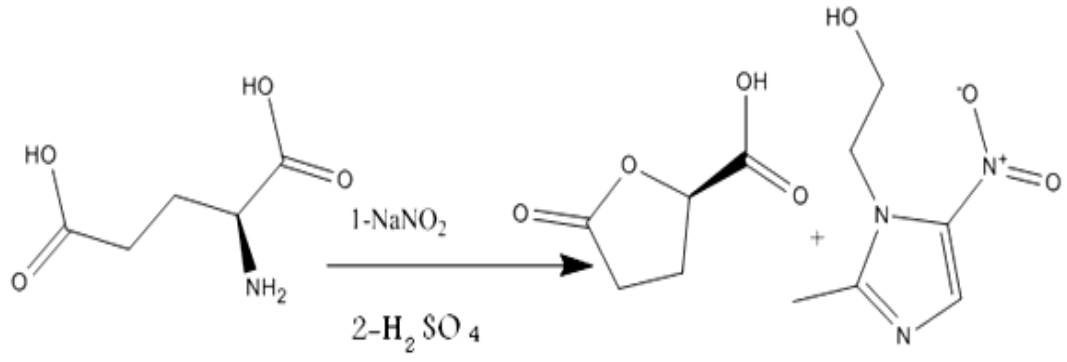
Isolates were cultured by streaking technique on blood agar plates, the Bacitracin and Optochin disks were put, the dishes were incubated in 37°c for 24 hrs. the appearance of non-growth halo around the disks indicating the positive results

6. Experimental:

General.Melting point was determined in open capillary tube on shimadzu,Japan melting point apparatus.The IR specta was recorded in KBr with a 1330 perkin-Elmer spectrophotometer.TLC plates in n-butanol:acetic acid :water system(50:11:25).The spot was visualized by exposing dry plate in iodine vapours.

7. Preparation of 2-furancarboxylic acid,tetrahydro-5-oxo-ɣ-Butyra lactone- ɣ-carboxylic acid.

ASolution of sodium nitrate(14.8gm,0.162 moles)in distilled water(35 ml)and sulfuric acid(75 ml,2N) were added dropwise simultaneously from separatory funnels to a suspension of L-glutamic acid(21.142gm,0.143 moles)in distilled water 55ml under vigorous stirring,After the addition is complete,The clear solution was stirred at room temperature for an additional 16 hr and water is removed under reduced pressure with rotary evaporator.The resulting pasty solid is triturated with boiling acetone(80 ml)and the hot solution is removed by decantation and set aside to cool.This operation is repeated four times.Removal of solvent with a rotary evaporated to give slightly yellow oil[13].The viscous oil was crystallized twice from ethyl acetate-benzene-petroleum ether(1:1:1)[14,15,16].The following reaction was explained as below.



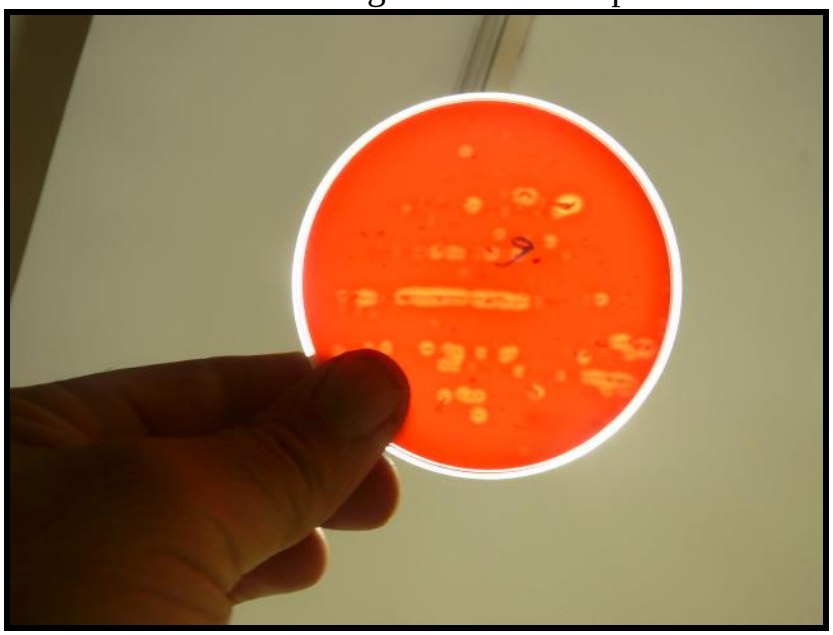
Glutamic acid compound A

$C_5H_6O_4$.. Yield:14.8 gm(62%);yellow powder;M.Wt.130.10;M.P 74-76Ċ;Rf 0.51 by using n-butanol:acetic acid:water(50:11:25) as solvent system;IR Spectrophotometer in (cm^{-1}):1775 (C=O) stri.vib. of lacton,1723(C=O) stri.vib. of acid.,1419 and 1250

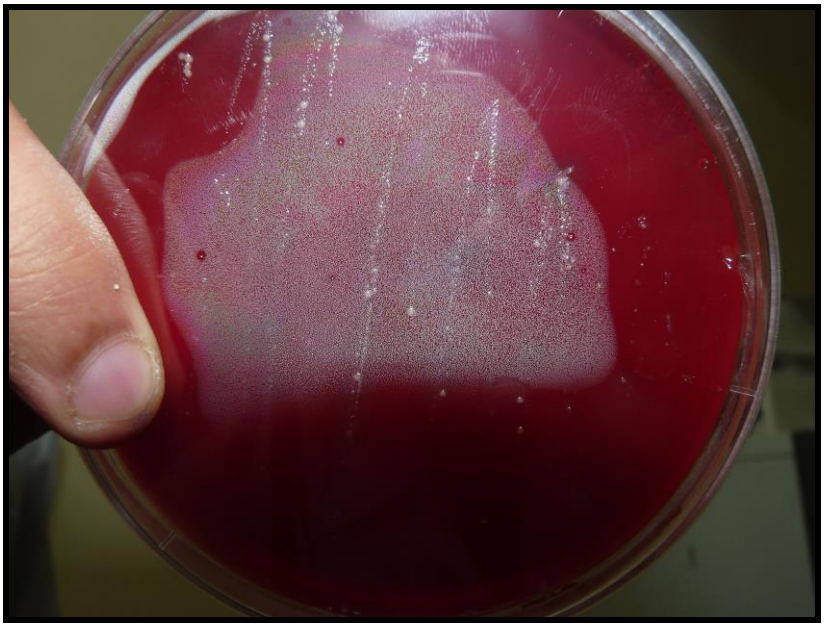
due to coupling between in plane (OH) Ben.vib. and (C-O) str.vib.,1201 and 1129 asym. and sym. C-O-C str.vib. of lacton.

Results and discussion

The isolates appeared as pin point colonies with a translucent circle of hemolysis (β-hemolysis) around them on blood agar as shown in pic.1. and Pic.2.



Pic.1. The β- hemolysis around the isolates



Pic. 2. The morphology of colonies on blood agar

The biochemical tests showed the presence of 3 isolates of ***S. pyogenes*** from 10 isolates submitted to the tests as shows in table (1)

Table 1: The biochemical tests of the isolates

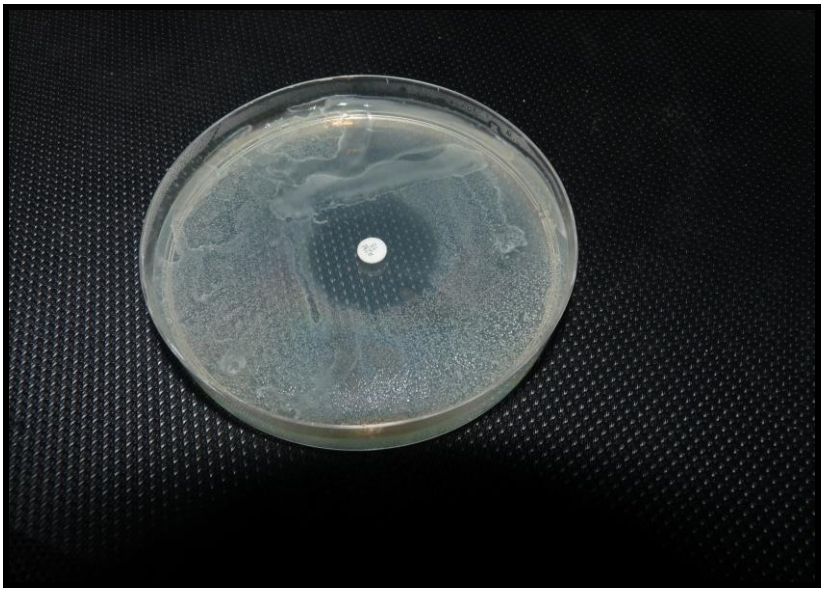
Isolate No.	Gram stain	morphology	haemolysis	DNase	oxidase	catalase	Basi	optoch
١	+	Chain	β	+	+	-	S	R
2	+	Chain	β	+	+	-	S	R
3	+	Chain	β	+	+	-	S	R
4	-	Cluster	β	-	-	+	R	R
5	-	Cluster	β	-	-	+	R	R
6	-	Cluster	β	-	-	+	R	R
7	-	Cluster	β	-	-	+	R	R
8	-	Cluster	β	-	-	+	R	R
9	+	Chain	α	-	-	+	R	R
10	-	Cluster	β	-	-	+	R	R

Pic.3.Sensitivity of the isolates to Bacitracin and optchin discs

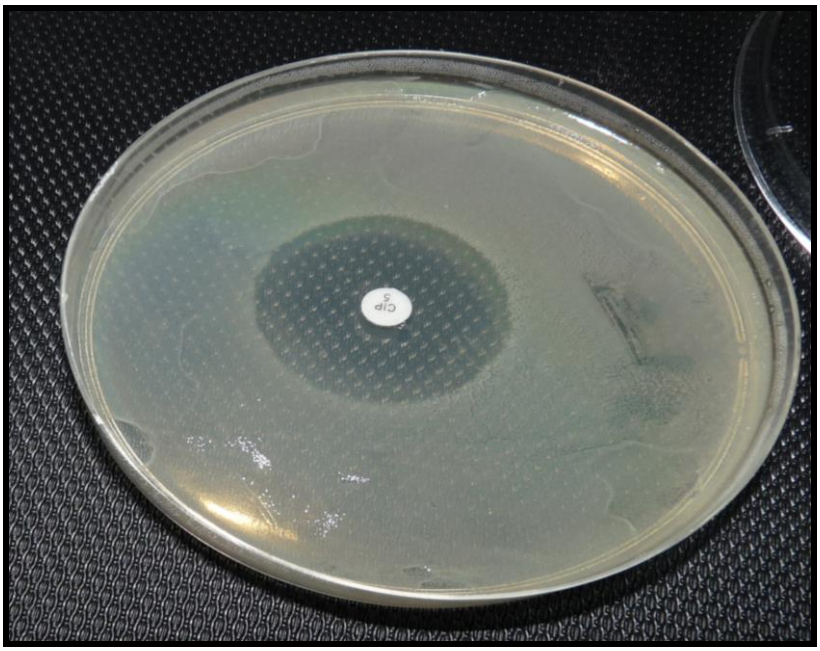
Pic.4. The interaction zone in DNase test

The ratio of isolation was (30%), a bit higher of what was mentioned by [17,18] . that is perhaps due to the difference of the studied age groups. In present study, the most patients were children, the age category that recorded the highest frequency infection of ***S. Pyogenes,*** or may be the studied patients were not treated with antibiotics that would inhibit the growth of bacteria.

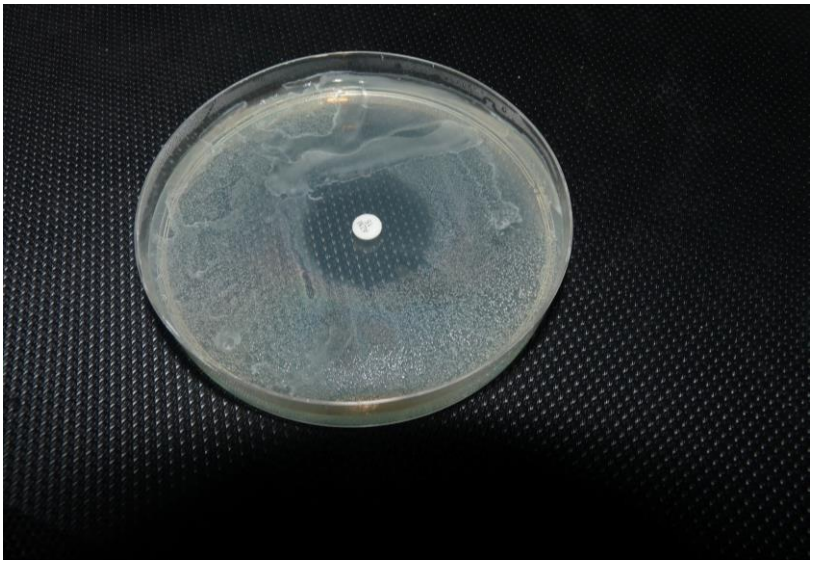
All the isolates gave a sensitivity to the three used antibiotics as showed in pics.5;6;7;8 and table 2 .



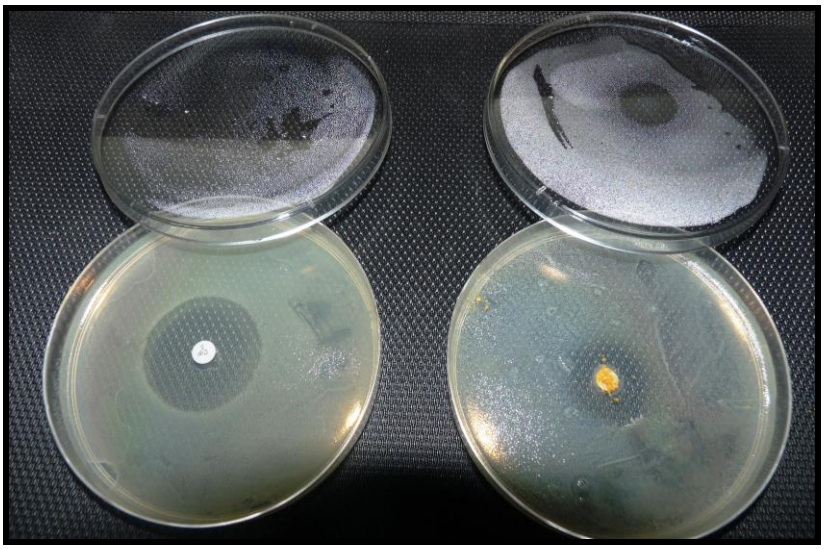
Pic.5. The inhibition zone of the AMX



Pic.6.The inhibition zone of CIP



Pic.7. The inhibition zone of the AZM



Pic8.The inhibition zone of the prepared compound(A)

Table 2 .The diameters of the in hibition zone of the three antibiotics and the prepared compound (A).

Antibiotic / The clinical isolate	AZM (mm)	AMC (mm)	CIP (mm)	(mm) Compound(A)
S. pyogenes	28	34	34	26
S.aureus	22	14	3	34
S.pneumonia	25	36	29	16

AZM= Azithromycin, AMC= Amoxicillin, CIP=Ciprofloxacin and compound (A)= 2-furancarboxylic acid,tetrahydro-5-oxo-ɣ-Butyra lactone- ɣ-carboxylic acid.

These results were expected due to the activity of these antibiotics to the bacteria ,amoxicillin is a moderate antibiotic has broad spectrum which inhibit the synthesis of the bacterial cell wall [19] , while the azithromycin is a very important antibiotic in tonsillitis , sore throat treatment and the sinusitis this antibiotic interferes with the protein synthesis , another important antibiotic choice is ciprofloxacin because of its ability in interference with the enzymes that recombine the DNA strands during the replication which blocks the protein and DNA synthesis [20] .
2-furancarboxylic acid,tetrahydro-5-oxo-ɣ-Butyra lactone- ɣ-carboxylic acid was shown to inhibit the growth of bacteria because furan ring and carboxyl groupe are presene in this compound .

Glutamic acid(2s)-2-aminopentanedioic acid,also referred to as glutamate(the anion),is one of the proteinogenic amino acids.It is not among the essential amino acids.[21]
Glutamic acid is present in a wide variety of foods and is responsible for one of the five basic tasted of the human sense of taste called(umami),especially in it is physiological form, the sodium salt of glutamate in a neutral PH[22].Ninty-five percent of the dietary glutamate is metabolized by intestinal cells in a first pass[23].

References:

1- Katrina P.Arguelles,Regina M.Naguit and Esperanza C.Cabrera."Incidence of streptococcus pyogenes as pharyngeal flora among children age 5 to 12 of barangay 708,leveriza,malate,manila(2004)33(4):149-151.

2- Theresa L.Lamagni,Shona Neal,Georgia Duckworth and Androulla Efstration"predictors of death after severe streptococcus pyogenes infection(2009).vol.15.No.8.

3- Dumre SP,Sapkota K,Adhikari N,Acharya D,Karki M,Bista,Basnyat SR,Joshisk .Asymtomatic throat carriage rate and anti microbial resistance pattern of streptococcus pyogenes in Nepalese school children.Kathmandu university medical journal(2009)vol.7,No.4.

4- Hawser P,Robert E,Susceptibility of Gram-Negative pathogens isolated from patients with complicated intra abdominal infections in united stes,2007-2008:results of the study for monitoring antimicrobial resistance trends(smart)(2010)vol.54.No.7.

5- Leni Mathew,X National pediatric Haematology-oncology conference (2006)vol.73.Indian Journal of pediatrics.

6- Arun Bansal,Sunit C,Singhi and M.Jayashree"penicillin and gentamicin therapy VS amoxicillin clavulanate in severe hypoxemic pneumonia(2006)vol.73.

7- Glaxosmithkline"augmentin ES-600(amoxicillin)clavulanate potassium powder for oral suspension(2009).

8- Falagas ME, Vouloumanou EK, Matthaiou DK, et al. Effectiveness and safety of short-course vs long-course antibiotic therapy for group A beta hemolytic streptococcal tonsillopharyngitis: A meta-analysis of randomized trials. Mayo Clin Proc(2008)83:880-889.

9- J.C.S.De Azavedo,R.H.Y Eung,DJ.Bast,CL.Duncan,S.B.Borgia and D.E.Low"prevalence andmechanisms of macrolide resistance in clinical isolation of group A streptococci from outario,Canada (1999).vol.43.No.9.p2144-2147.

10- Matteo Bassetti,Graziana manno,Andrea collida,Alberto ferrando,Giorgio gatti et al"Erythromycin resistance in streptococcus pyogenes in Italy(2000)vol.6,No.2.

11- Pavolovic Annual Research Review et al."streptococcus pyogenes is a live and well"(2009)vol.51.No.3.p122-127.

12- Shulman ST,Ayoub EM.Poststreptococcal reactive arthritis.Curr Opin Rheumatol (2002)14:562-565.

13- Carapetis JR,MC Donald M,Wilson .N.J.Acute rtheumatic fever.lancet (2005),366:155-168.

14- Clayton,J.P,et al ,Lacton Esters of penicillins,J.Med.Chem.(1976)vol.19,Issue 12,p.1385-1319.

15- Gringone,O.H.of rouessac,F.P.(S)-(+)- ɣ-butyrolacton,organic syntheses(1990) vol..7p.99.

16- Silverstein,M.et al,synthesis of the Enantiomers of lactones with knowa absolute configuration,tetrahydron(1978)vol.34.p.1449.

17- Mori,K,Synthesis of optically active forms of sulcatol,tetrahydron(1975)vol.31.p3011-3012.
18- National committee for clinical laboratory standards for antimicrobial susceptibility testing:fourteen information supplement,aproned standard,NCCLS Document100-s14.vol.24No.1(2004).
19- Swanson-biearman B,Dean BS,Lopez G,Kreu Zelok EP.The effect of penicillin and cephalosporin ingestions in children less than six years age.vet Hum Toxicol (1988),30:66-67.
20- Pinho, M. D., Melo-Cristino, J., Ramirez, M., and the Portuguese Group for the Study of Streptoc, Fluoroquinolone Resistance in Streptococcus dysgalactiae subsp. equisimilis and Evidence for a Shared Global Gene Pool with Streptococcus pyogenes. *Antimicrob. Agents Chemother*(2010) 54: 1769-1777.
21- Gornier,R,et al,Thioproline,The Lancet,Feb.16th(1980),p68-70.
22- Mackenzie,C.and Harris,J,N-Formylcysteine synthesis in Mitochondria,J.Am.Chem.SOC,(1956)VOl88,No.2,p393-406.
23- Mitochondria,J.Am.Chem.SOC,(1956)VOl88,No.2,p393-406.
White,A,Handler,P,Smith,E,Principles of biochemistry, (1990),9th ED,P.90-92
Ikeda,K,Umami,J.Chem,Soc.Tokyo (1909)vol.3,p.820-
836.White,A,Handler,P,Smith,E, Principles of biochemistry, (1990) ,9thED,P.95.