Effect of Histidine on Sensetivity of Some Pathogenic Bacteria

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Abstract

The purpose of this study was to determine the antibacterial effect of the amino acid histidine against two pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*. No antibacterial activity was observed for histidine at all tested concentrations. The sensitivity test for *E.coli* and *S. aureus* was performed against ten types of antibiotics. The antibiotics used for *E.coli* were Levofloxacin(Lev), Streptomycin(S), Rifampicin(Rp), Metronidazole(Mz), Cloxacillin(Cx), Ciprofloxacin(Cip), Tetracycline(T), Nalidixic acid(Na), Penicillin(P) and Vancomycine(Va) and the results of inhibition zones were 44, 14,8, 0,0, 35, 24, 28, 16 and 14 mm respectively. When the Histidine added at concentration 10 mg/ml to Mueller hintone agar, the inhibition zones became 24, 12, 18, 0, 0, 24, 32, 25, 13 and 14 mm for each antibiotic respectively. The antibiotics used for*Staph. aureus* areLevofloxacin (Lev), Gentamicin(Gm), Rifampicin(Rp), Metronidazole(Mz), Cloxacillin(Cx), Ciprofloxacin(Cip), Tetracycline(T), Clarithromycin(Cla), Vancomycine (Va) and Chloramphenicol (C). Result of inhibition zones were 40, 30, 0, 0, 0, 22, 10, 0, 10 and 22 mm when the Histidine added at concentration 10 mg/ml to the mueller hinton agar, the inhibition zones became 30, 20, 16, 0, 0, 26, 15, 0, 10 and 22 mm. Applicable experiment of the histidine effect was conducted on resistant and sensitive isolates against Histidine of *Mycobacterium tuberculosis* (TB) in Pulmonary Institute in Baghdad. It was found that mixing of Histidine with Rifampicin changed the response of resistant isolate against Rifampicin and turned into sensitive. This results may encourage mixing antibiotics and Histidine instead of using the more effective and harmful antibiotics against the resistant isolates.

Keywords: Histidin, Pathogenic bacteria, Susceptibility test, Rifampicin.

Introduction

Histidine, an essential amino acid, has a side chain of positively charged imidazole functional group. The imidazole group makes it a common participant in enzyme catalyzed reactions. Unprotonated imidazole is nucleophile and can serve as a general base, while the protonated form can serve as general acid. The residue can also serve a role in stabilizing the folded structures of proteins [1]. The amino acid L-histidine (His) was discovered independently by Kossel and Hedin in 1896 [2]. The histidine has antibacterial activity[3]. Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase [4]. Rifampicin binds to RNA polymerase at a site adjacent to the RNA polymerase active center and blocks RNA synthesis by physically blocking the formation of the phosphodiester bond in the RNA backbone, preventing extension of RNA products beyond a length of 2-3 nucleotides ("steric-occlusion" mechanism) [5,6].

Tuberculosis treatment refers to the medical treatment of the infectious disease tuberculosis (TB). The Rifampicin use in the first line of treatment of tuberculosis. Rifampicin is indicated in adults and children 12 years and older for the treatment of active pulmonary tuberculosis caused by *M. tuberculosis*. Rifampicin must always be used in combination with one or more antituberculosis drugs to which the isolate is susceptible [7,8]. In many countries, the wide use of the standard short-course regimen has led to an increasing incidence of multidrug-resistant (MDR) tuberculosis (TB), defined as resistance to at least isoniazid (INH) and Rifampicin. Mutations in rpoB, the gene encoding the β subunit of DNA-dependent RNA polymerase, are associated with Rifampicin resistance in *Mycobacterium tuberculosis* [9]. Drug susceptibility of *M. tuberculosis* can be determined either by observation of growth or metabolic inhibition in a medium containing antituberculosis drug, or by detection, at the molecular level, of

mutations in the genes related to drug action [10].

This Study aimed to screen the effect of Histidine as antimicrobial agent and interaction with antibiotics against pathogenic bacteria *E.coli*, *Staph. aureus* and TB isolates.

Materials and Methods

Microorganisms:

Two isolates of bacteria were used in this study including *E. coli* and *Staph. aureus*. These isolates were supplied by department of medical biotechnology/college of applied biotechnology. The isolates were re-purified and activated in enrichment and selected media (Eosin Methylene Blue, MacConky agar, Nutrient broth, Nutrient agar, Mannitol salt agar). These media were prepared according to the manufacturing company (Hi-media).

Histidine stock solution:

It was prepared at a concentration of 100 mg/ml in deionized water and was sterilized using Millipore filter (0.22 µm).

Histidine medium:

It was prepared by adding histidine at concentrations 0.1-10mg/ml in Muellerhintone agar. Aliquot of 25 ml of prepared medium with histidine was poured in petridishes for each concentration.

Antimicrobial susceptibility test was conducted using discs diffusion technique by distributionon the surface of the medium as shown in Table (1). These antibiotics were selected for each isolate according to the resistance and sensitive response of these isolates [12,13].

Table (1)
Standards susceptibility tests of E. coli and Staph. aureus to the antimicrobial agents.

Bactrial isolate	*Antimicrobial*	
	Resistant to	*Sensitive to*
E. coli	Vancomycine Streptomycin Metronidazole Cloxacilline Rifampicin Penicillin	Tetracycline Levofloxacin Ciprofloxacin Nalidixic acid
Staph. aureus	Metronidazole Cloxacilline Clarithromycin Rifampicin Tetracycline	Vancomycine Gentamycin Levofloxacin Ciprofloxacin Chloramphenicol

***M. tuberculosis* (TB) response to histidine:**

The response of TB to the histidine was conducted in TB institute, Ministry of Iraqi health. The examination was done using the standard steps recommended by the World Health Organization and as follows [11]:

After characterization of the isolates as TB using ideal medium Lowenstein-Jensen medium (LJ medium), two concentrations of Histidine 5 and 10 mg/ml were added to LJ-medium containing Rifampicin 40µg/ml. Previously identified TB isolates as Rifampicin -sensitive and resistance were used to inoculum these media. The inoculums of isolates were prepared by the staff of TB Institutes according to the standard procedure [11]. After inoculums, it was added to the media incubated at 37 ^{o}C for 30- 40 days.

Results and Discussion

Effect of histidine:

There was no effect of free Histidine at all concentrations used on the growth of *Staph. aureus* and *E.coli* and this result is not in agreement with Kacprzyka *et al* [3], who referred to the act of histidine as an antimicrobial agent.

Susceptibility test:

Table (2) showed the susceptibility test of *E.coli* against antibiotics in presence of serial concentrations of histidine ranged from 1 to 10 mg/ml in comparison with the control treatment without histidine and the presence of antibiotics. Control treatment illustrated the sensitivity of *E. coli* against Levofloxacin, Ciprofloxacin, Tetracycline, Vancomycine, Streptomycin and Nalidixic acid and resistant to Metronidazole, Cloxacilline and Penicillin according to [12]. There was no effect of His at different concentrations on the response of *E. coli* against (Vancomycine, Streptomycin, Metronidazole, Cloxacilline, Nalidixic acid and Penicillin). The sensitivity against Rifampicin and Tetracycline was increased and reduced against Levofloxacin and Ciprofloxacin starting from 1 mg/ml Histidine. For *Staph. aureus* (as shown in Table (3)), It was found the resistance of bacteria against Metronidazole, Clarithromycin, Rifampicin, Cloxacilline, Tetracycline and penicillin[12]. The sensitivity varied in presence of Histidine

and increased for Rifampicin (> 3mg/ml), Tetracycline (>1 mg/ml) and reduced for Levofloxacin (>1mg/ml) and Gentamycin (>1 mg/ml). No change in the sensitivity was found against Vancomycine, Chloramphenicol, Cloxacillin, Metronidazole and Clarithromycin. Interpretation change of the resistance and sensitivity to antibiotics may be due to influence of synergistic or antagonistic of Histidine with antibiotics. In the case of Rifampicin and Tetracycline, there was an increase in the sensitivity for each isolates and the antagonistic interactions appeared in reducing the efficiency of antibiotic.

Table (2)
Effect of the serial concentration of histidine on susceptibility of E. coli.

Type of Antibiotic	*Concentration of Histidine (mg/ml)* / *Diameter of Inhibition zone (mm)*										
	control	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Streptomycin (S 10)	14	22	20	20	19	20	20	18	17	14	12
Rifampicin (Rp 5)	8	11	12	11	12	13	13	13	13	14	18
Metronidazole (Mz 5)	0	0	0	0	0	0	0	0	0	0	0
Cloxacillin (Cx 5)	0	0	0	0	0	0	0	0	0	0	0
Levofloxacin (Lev 5)	44	35	40	36	40	37	31	26	22	23	24
Ciprofloxacin (Cip 5)	35	25	25	23	26	20	22	25	24	24	24
Tetracycline (T30)	24	35	30	28	25	32	30	30	27	30	32
Naldixic acid (Na30)	28	24	28	28	30	20	20	22	25	26	25
Pencillin (P 10)	16	14	14	13	14	13	12	13	13	13	13
Vancomycin (Va30)	14	14	14	13	14	12	13	14	12	13	14

Table (3)
Effect of the serial concentrations of histidine on susceptibility of Staph. aureus.

Type of Antibiotic	*Concentration of Histidine (mg/ml)* / *Diameter of Inhibition zone (mm)*										
	control	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Metronidazole (Mz5)	0	0	0	0	0	0	0	0	0	0	0
Vancomycin (Va30)	10	10	10	10	12	10	10	10	10	10	10
Cloxacillin (Cx5)	0	0	0	0	0	0	0	0	0	0	0
Clarithromycin (Cla15)	0	0	0	0	0	0	0	0	0	0	0
Rifampicin (Rp5)	0	0	0	8	12	15	16	15	16	15	16
Gentamicin (Gm120)	30	30	27	30	27	20	20	23	20	23	20
Levofloxacin (Lev5)	40	35	40	35	30	25	25	27	30	25	30
Ciprofloxacin (Cip5)	22	22	24	26	28	26	25	26	25	24	26
Tetracyclene (T 30)	10	13	14	14	14	13	15	15	15	15	15
Cloramphinicol (C30)	22	21	21	21	21	21	21	21	21	21	21

Effect of Histidine on response of TB

Tables (2) and (3) exhibit clear that the response to the Rifampicin is increasing for *E.coli* and *Staph. aureus* at low concentrations of Histidine (>1 and >3 mg/ml respectively). In cases of Multidruge resistant isolates (MDR), the common effective antibiotic for examination the sensitivity is the isonized with the same procedure of the test with Rp [13]. The use of histidine at concentration 5 and 10 mg /ml with Rp altered the response of resistant isolate to the Rp and became sensitive. Fig.(1), a and b shows the response of sensitive isolate of TB to Rp supplemented

with 5 and 10 mg/ml of histidine. The change in response of the resistant isolates may be due to the interaction of Rp with Histidine and invaded anthor targets in the β subunit of DNA-dependent RNA polymerase responsible for most of the variation in Rp resistance appeared or may the Histidine played the defect complement in reverse the mutant isolate. These new hypothesis needs more investigation at the molecular level.

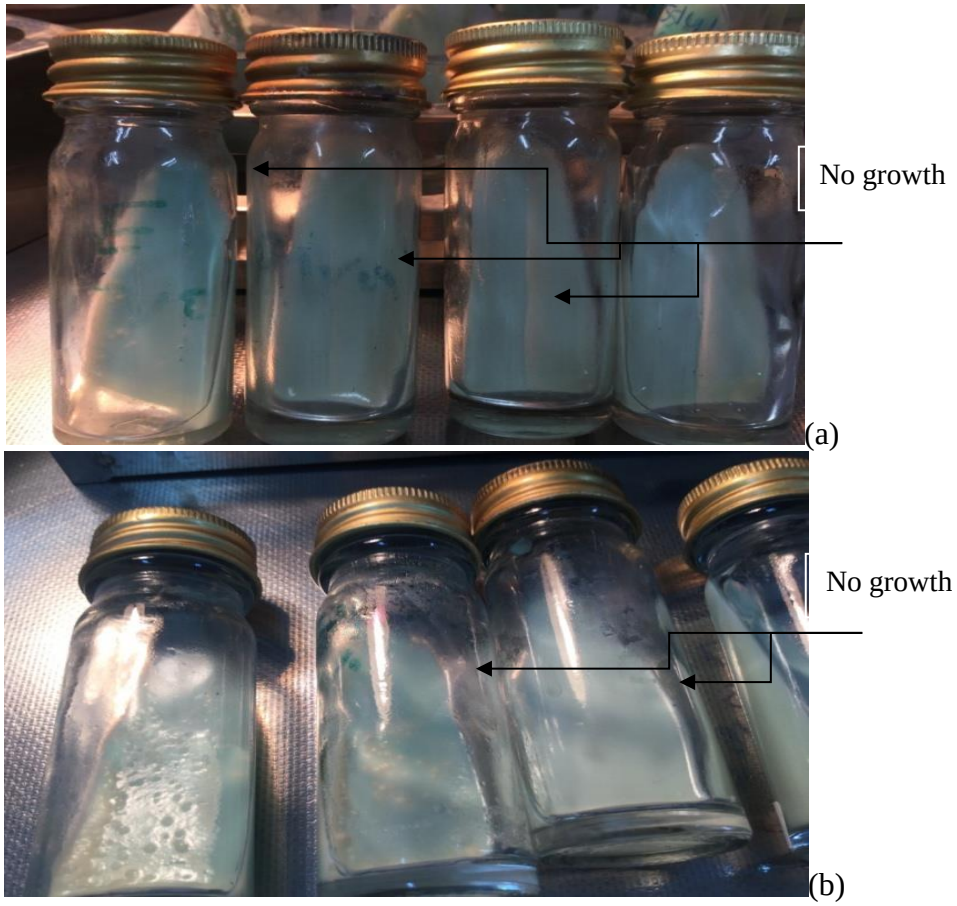

Fig.(1): Growth of Mycobacterium tuberculosis in LJ-medium in presence of Histidine. a, Rifampicin sensitive isolate; b, Rifampicin resistant isolate.

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الخلاصة

الهدف من الدراسة الحالية هو لمعرفة تأثير الحامض الاميني الهستدين على استجابة نوعين من البكتريا المرضية *Escherichia coli* و *Staphylococcus aureus*. لم يلاحظ تأثير مباشر لإضافة تراكيز مختلفة من الحامض الاميني الهستدين على كلا النوعين من البكتريا. تم اجراء فحص الحساسية على نوعي البكتريا باستعمال عشرة انواع من المضادات الحياتية لكل نوع من انواع البكتريا اذ استعملت المضادات التالية لل *E.coli* وهي (ليفوفلوكساسين, ستريبتومايسين, ريفامبسين, ميترانيدازول, كلوكساسلين, سبروفلوكساسين, تيتراسايكلين, نالديكسك اسيد, بنسلين, فانكومايسين) وكان قطر هالة التثبيط على التوالي هو ٤٤, ١٤, ٨, ٠ ,٠, ٣٥ , ٢٤ , ٢٨ , ١٦ , ١٤ ملم وبعد اضافة الحامض الاميني الهستدين الى الوسط الزرعي مولر هينتون بتركيز ١٠ ملغم لكل مل لوحظ تغيير قياس هالة التثبيط ٢٤, ١٢ , ١٨ , ٠ , ٠, ٢٤ , ٣٢ , ٢٥ , ١٣, ١٤ ملم. اما بالنسبة لل *Staph. aureus* فقد تم استعمال المضادات التالية ليفوفلوكساسين, جينتامايسين, ريفامبسين, ميترانيدازول, كلوكساسلين, سيبروفلوكساسين, تيتراسايكلين, كلاريثرومايسين, فانكومايسين, كلورامفينيكول وكان قطر هالة التثبيط على التوالي هو ٤٠ , ٣٠ , ٠ ,٠, ٠ , ٠ , ٢٢ , ١٠, ١٠، ٠ , ٢٢ ملم وبعد اضافة الحامض الاميني الهستدين الى الوسط الزرعي مولر هينتون اكار بتركيز ١٠ ملغم لكل مل لوحظ تغيير قطر هالة التثبيط الى الاتي ٣٠ ، ٢٠ , ١٦ , ٠ , ٠ , ٢٦ , ١٥ ، ٠ , ١٠ , ٢٢ ملم. بعد ذلك انجزت التجربة التطبيقية على عزلات حساسة ومقاومة للريفامبسين لبكتريا السل *Mycobacterium tuberculosis* في معهد التدرن الرئوي في بغداد. وجد ان خلط الهستدين مع المضاد قد غير من استجابة العزلة المقاومة وتحولت الى حساسة. هذه النتائج قد تشجع على عدم استعمال مضادات حياتية اكثر فعالية وضارة ضد العزلات المقاومة.