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RESEARCH ARTICLE

The Tolerability and Degradation Rate of Amoxicillin Alone and with Clavulanic Acid in the Acidic and Alkaline Medium

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Abstract

This study deal with the effects of various pH values (1-14) on the stability of Amoxicillin alone and with Clavulanic acid. The degradation rate of Amoxicillin alone and with Clavulanic acid was determined by using *Bacillus thuringiensis* as indicator to act of drug used in this study. Amoxicillin alone and with Clavulanic acid was high stability at an acidic pH, while increasing the pH value led to decrease its stability at the statistical level (P value < 0.05). Maximum stability of Amoxicillin alone and with Clavulanic acid was experienced at pH values (4, 5,6,7,8 and 9), While their degeneration rate was significantly higher at acidic medium (pH=1) and alkaline pH medium (pH= 11, 12, 13 and 14) at p-value < 0.05. Therefore, the Amoxicillin alone and with Clavulanic acid is best given to humans and animals in pH value from 4-9.

Key words: *Tolerability, Degradation, Amoxicillin, Clavulanic, acid, Alkaline, Medium.*

Introduction

Amoxicillin drug is a ß-lactam antibiotic and it is classified as antibiotic in a group of drugs called Penicillin [1].Clavulanic acid is a ß-lactamase inhibitor substance produced by *Streptomyces clavuligerus* [2].Combination of Amoxicillin-Clavulanic acid is considering one of the famous drug as used antibiotics worldwide[3] and these combinations are commonly prescribed for bacterial infections in the urinary tract, otitis media, and respiratory tract infections [4].

One way to give Amoxicillin is by oral and the acidity of the gastric is the first challenge facing these drugs (Amoxicillin alone and with Clavulanic acid) .Gastric acid is a fluid composed mainly from HCl, and it is very important to digest foods proteins and this acidity being maintained by the proton pump which called H^+/K^+ ATP-ase.

In the human stomach, the pH of gastric acid is 1.5 to 3.5 [5]. When giving an oral medication will be affected by several factors, such as digestive juices, the duration of survival in the stomach, and the chemical properties of the drug, for that this study aimed to determine the effects of different potential hydrogen (pH 1 till 14) on the activity of Amoxicillin alone and with clavulanic acid when taken by oral route.

Materials and Methods

Parameters of Study

The Parameters of this Study Include:-

- The Antibacterial activity (Inhibition zone diameter) of Amoxicillin alone and with Clavulanic acid after mixing with the acidic and alkaline solution at pH values from 1 till 14.
- 2-Tolerability and degradation of Amoxicillin alone and with clavulanic acid after mixing with the acidic and alkaline solution at pH values from 1 till 14.

After the preparation of previous solutions incubated for 4 hours [The period in which the food stays in the stomach is 4 hours) [6, 7] at a temperature of 37°C

Microbe's Isolates

Bacillus thuringiensis (Model of G+ Bacteria) isolate was taken from Bank of Technical institute Babel/Microbiology Lab. and was used as an indicator of antibacterial activity of the drugs used in this study.

Bacillus Thuringiensis Counts

Counts of the Bacteria (***Bacillus thuringiensis***) was done by using colometeric methods (Spectrophotometric method) by adding five ml of sterile normal saline to colonies of ***Bacillus thuringiensis*** (Aged 24 hours at 37ºC) which cultured on the nutrient agar media and mixed well with colonies then calculated the turbidity solution (Result from normal saline mixed with harvested bacteria) by serial test tube and adjusted accordance to the absorbance of 0.08-0.1 at 625nm corresponding to 10^8CFU/ml NCCLS [8].

Preparation of Acidic and Alkaline Solution

Acidic stock solution (5%) was prepared by dissolving 5 ml of HCl **(37%)** in the 100 ml distilled water.

Alkaline stock solution (4%) was prepared by dissolving 4 gm NaOH in the 100 ml of distilled water.

Both stock solutions used to prepare other solutions with different pH values (from 1 till 14) by using pH meter.

Preparation of Drug Concentrations

In this study, concentrations of drugs and solutions used were prepared as follows**:-**

Group A (Before antibacterial added), in this group, all solutions were prepared (with pH values from 1 to 14) without added amoxicillin or Clavulanic acid.

Group B was prepared by taking 0.5 ml from stock solution contained 100ml of oral suspension drug (Glomox®, manufacture in Globalpharma Co. L.L.C. Dubai.UAE, each five ml of this stock contained 250mg of amoxicillin trihydrate) and added to 49.5 ml for each solution at pH values from 1 to 14 to produce final concentration 0.5 mg/ml).

Group C was prepared by taking 0.5 ml from stock solution contained 60ml of oral suspension drugs (Amoksiklav®, manufacture by medica. Bahi company /Damascus-Syria, each five ml of this stock contained 250mg of Amoxicillin trihydrate and 62.5 mg of Potassium Clavulanate) and added to 49.5 ml for each solution at pH values from 1 to 14 to produce final concentration 0.5 mg/ml of Amoxicillin trihydrate and 0.125mg/ml of Potassium Clavulanate).

Groups D (Control positive group) was prepared by taking 0.5 ml from stock solution of group B and C and added to 49.5 ml of distilled water to produce final concentration 0.5 mg/ml Amoxicillin trihydratewhich represent Control positive for group B and at the same time, considered the concentration 0.5 mg/ml of Amoxicillin trihydrate /0.125mg/ml of Potassium Clavulanate) as Control positive for group C.

Group E (Control negative group) distilled water was added only to the well (Well diffusion method).

All previous groups placed in the incubator for 4 hours at 37°C before starting to examine its antibacterial action.

Bacillus thuringiensis Sensitive Test

According to the procedure of bacterial sensitive test, One hundred µl were taken from ***Bacillus thuringiensis*** standard solution (10^8 CFU/ml) and spread uniformly over and Nutrient agar media by using the spreader, then left for one hour to dry of cells on the media surface. By using cork borer, three wells were worked on the cultured media. Five microliter was taken from each Group (A, B, C and D) that has been prepared previously and put in these wells (Each well filled with one of the materials that have been studied).

Same previous steps were used again for distilled water which considered as control negative group (Group E). Number of petri-dish for each group repeated 5 times and each petri-dish contains three holes. Inhibition activities of Amoxicillin trihydrate alone and with Potassium Clavulanate that present in different pH degree solution (acidic and alkaline phase [pH 1 till 14]) were determined by measuring the zones inhibition formed around the well by millimeter unit. The plates were observed for presence of zones of inhibition around the after 24 hours at 37ºC [9].

Potential hydrogen (pH) tolerability test

Aimed this test to determine of tolerability and degradation of Amoxicillin alone (group A) and with Potassium Clavulanate (Group

B), when mixed with various solutions pH values from 1-14.

Potential hydrogen tolerability test divided into three tests:-

First test:-The Antibacterial effect of HCl and NaOH on the ***Bacillus thuringiensis.***

This test aimed to determine the effects of different pH values(1 till 14) solutions on the ***Bacillus thuringiensis*** growth by using well diffusion method after incubate for 24 hours at 37ºC (This test include Group A).

Second test :-The Antibacterial activity of Amoxicillin trihydrateafter mixed with different pH values (1 till 14) solutions on the ***Bacillus thuringiensis*** growth by using well diffusion methods through cultured on the nutrient agar after incubate for 24 hours at 37ºC (this test include Group B).

Third test :-The Antibacterial activity of Amoxicillin trihydratewith /Potassium Clavulante after mixed with different pH values (1 till 14) solutions on the ***Bacillus thuringiensis*** growth by using well diffusion methods through cultured on the nutrient agar after incubate for 24 hours at 37ºC This test include Group C).

Group D (Control Positive group) and Group E (Control Negative group) were used as comparative groups only.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS, version 15.0, Chicago, IL, USA) was used to Statistical analysis of the experimental results were conducted and one way ANOVA was used to assess the significance of changes between experimental groups . The data were expressed as Mean± Standard Errors (SE) and p-value<0.05 was considered statistically significance. LSD was carried out to test the significance levels among means of treatments.

Results and Discussion

The results of our study showed that different effects after added of Amoxicillin alone and with Potassium Clavulanatein the various pH values solutions (Acidic phase and Alkaline phase) solutions against ***Bacillus thuringiensis*** growth.

First test Aimed this test to detect the effects of HCl and NaOH on the ***Bacillus thuringiensis*** growth [Before added Amoxicillin alone and with Potassium Clavulanate in the various pH values (Acidic phase and Alkaline phase)].

The results of the first test showed No inhibition zone in all pH values (1-14) as in Figure 1, these results may have occurred because of:-

First Caused: - The amount the acidic or alkaline solution added was low (5 µl of acidic or alkaline solutions were added in each well in media used) and this amount is insufficient to influence the growth of the bacteria.

Second Caused:- The result of current study showed *Bacillus thuringiensis* having ability to tolerance to acidic and alkaline solution (pH 1-10), this result agree with [10] ,who studied the effects of 5% oxalic acid and 4% NaOH on the Pasturella, Klepsialla spp. ,*Escherichia coli*, Bacillus spp., the result of these study showed inhibit bacteria growth which has been studied except bacillus spp. on the other hand, the result of our study may be occurred because of Amoxicillin trihydrate is stable in acidic pH solutions, while increasing the pH values led to decreased its stability [11].

Fig.1: Well-diffusion method of first test for different pH values solution (pH value from 1 till 14), C⁻ = negative group (Distilled water only). Note: - Each number written on the surface of the Petri dish represents the value of the pH used

Second Test Aimed this test to detect the effects of acidic and alkaline solutions on the Amoxicillin trihydrate activity by the presence of bacteria evidence of the effectiveness of the antibacterial of above drug. The present study showed a significant increase (p-value <0.05) in Zone inhibition diameter between the experiment groups (At pH 1-10), while no inhibition zone diameter was noticed in the alkaline pH solution (11, 12,13and14), in comparison with the control group as in Table 1 and Figure 2.

Table1: The inhibition zone diameter of different pH values (1-14) solutions mixed with Amoxicillin alone against *Bacillus thuringiensis* (The age of colonies 24 hours at 37 ºC)

pH value	Inhibition zone (mm) M±SE
1	18.33± 0.55 A
2	23.50±0.76 BD
3	26.33±0.49 CD
4	24.00±0.73 D
5	25.83±0.40 C
6	27.50±0.61 D
7	27.66±0.49 CD
8	26.16±0.40 CL
9	20.33±0.42 E
10	24.60±0.61 D
11	00.00±00.00 F
12	00.00±00.00 F
13	00.00±00.00 F
14	00.00±00.00 F
C+	24.83±0.72 BD
C-	00.00±00.00 F

LSD=1.07 Different capital letters denote significant results (p-value <0.05) between different groups. C^+=Inhibition zone diameter result from distilled water mixed with Amoxicillin only. C^- =Control group (Distilled water only).

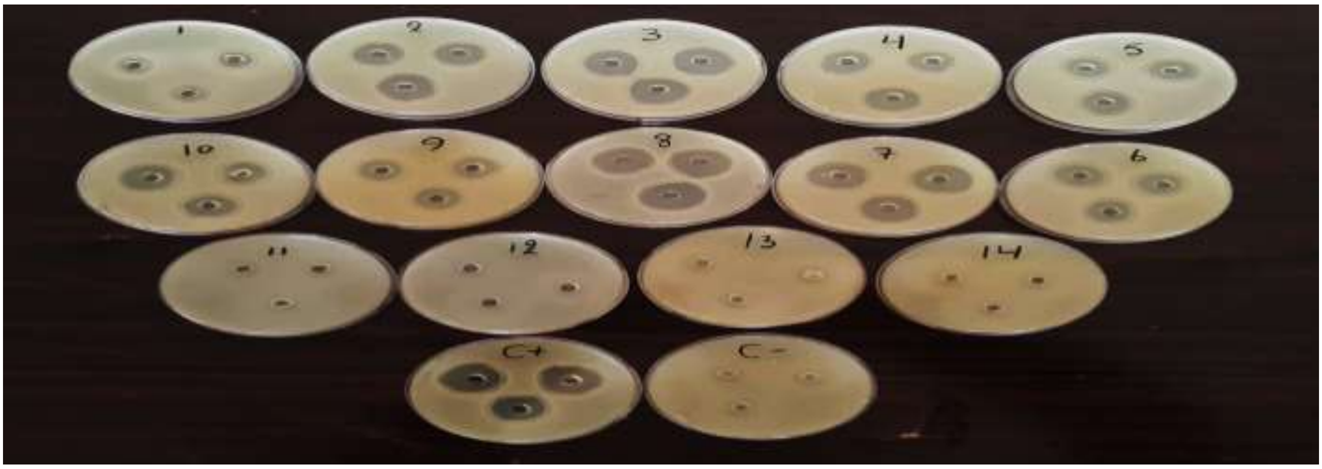

Fig.2: Inhibition Zone diameter (Well-diffusion method) of different pH value solution (pH value from 1 till 14) mixed with Amoxicillin as antibacterial against *Bacillus thuringiensis* in nutrient agar (Incubation period 24 hours at 37°C), C^+=inhibition zone diameter result from distilled water mixed with amoxicillin only. C^- =Control group (Distilled water only). Note: - Each number written on the surface of the Petri dish represents the value of the pH used

These results agree with [12], who referred to Amoxicillin stability is greatly reduced in highly acidic environment (stomach) and also in weakly. In other study [13], who noted that Amoxicillin is highly stable at the pH range of 4 - 8, and when pH<4 to 2, their stability decreased obviously due to that Amoxicillin was degraded via acid hydrolysis to Amoxicilloic acid. At pH=2 a stronger signal of Amoxilloic acid was also observed, it is caused by a mechanism involving the transient oxazolone structure. Otherwise, the response of Amoxicilloic acid increased with increasing pH value (from 2-8), and decreased slightly with increasing pH to 9 due to degradation of the Amoxicilloic acid. When pH<4 or pH≥9, the response of Amoxilloic acid observed due to degradation of Amoxilloic acid.

Third test:- Aimed this test to detect the effects of acidic and alkaline solutions on the ***Bacillus thuringiensis*** growth [After added Amoxicillin trihydrate / Potassium Clavulanate in the various pH values (Acidic phase and alkaline phase)].

Statistically significant differences (p-value<0.05) were found in the degradation rate of Amoxicillin trihydrate /Potassium

Clavulanate measured at pH 1 (Inhibition zone diameter was small) and more effects were showed in the pH 11, 12, 13, 14 (No inhibition zones diameter were noted) in compares with control group as in Table 2 and Figure 3.

Table 2: The inhibition zone diameter of different pH values solution mixed with Amoxicillin alone and with Potassium Clavulanate against *Bacillus thuringiensis* (The age of colonies 24 h days at 37°C)

pH value	Inhibition zone (mm) M±SE
1	17.33±0.95 A
2	21.00±0.85 BC
3	22.16±0.65 C
4	24.16±0.40 DG
5	26.83±0.60 EF
6	25.33±0.42 DF
7	25.60±0.49 EF
8	25.16±0.40 F
9	25.33±0.49 EFG
10	21.16±0.40 BC
11	00.00±00.00 H
12	00.00±00.00 H
13	00.00±00.00 H
14	00.00±00.00 H
C+	22.83±0.74 CD
C-	00.00±00.00 H

LSD=0.97Different capital letters denote significant results (p- value <0.05) between different groups. C^+=Inhibition zone diameter result from distilled water mixed with Amoxicillin only. C^- =Control group (Distilled water only)

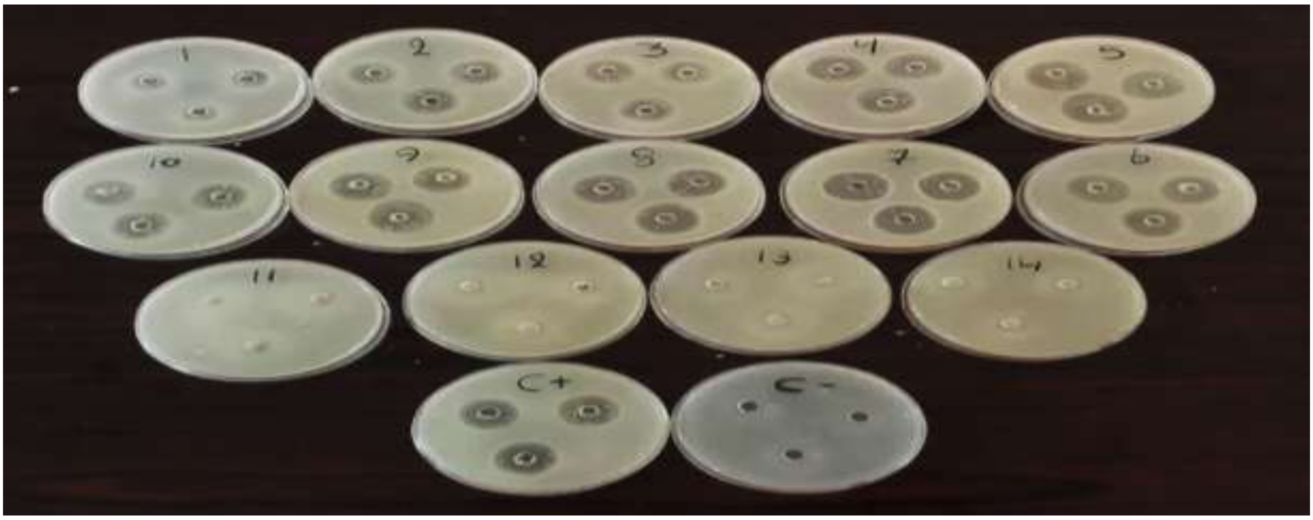

Fig.3: Inhibition Zone diameter (Well-diffusion method) of different pH value solution (pH value from 1 till 14) mixed with Amoxicillin and Potassium Clavulanateas antibacterial against *Bacillus thuringiensis* in nutrient agar (Incubation period 24 hours at 37°C), C^+=inhibition zone diameter result from distilled water mixed with Amoxicillin and Potassium Clavulanate. C^- =Control group (Distilled water only). Note: - Each number written on the surface of the Petri dish represents the value of the pH used

Potassium Clavulanate is less stable than Amoxicillin in the alkaline and acidic environment [14]. In a study conducted on the effects of different pH values on the degradation rate of Potassium Clavulanate and this study reached to found that acidic and alkaline environment significantly accelerated the decomposition rate of the substance as compared to neutral pH. [11].

In other study prepared by [15,16] were noted that the degradation rate of Amoxicillin at different pH values showed significant differences between pH 3 and 7, pH 3 and 10 , and pH 7 and 10.0 (p- value< 0.05). After 24 hours of Amoxicillin trihydrate remained active at pH values of 3, 7 and 10, respectively. Amoxicillin trihydrate proved to be the most stable in acidic environment, and increasing the pH slightly decreased its stability. Degradation was more pronounced at higher pH. In our study has shown that the Amoxicillin remain active from pH 1-10,While other pH values (11, 12, 13 and14) led to a complete breakdown of Amoxacillin.

Amoxicillin in both tests(2nd and 3rd test) was lost part of its anti-bacterial activity against *Bacillus thuringiensis* at pH 1 and complete loss at pH 11,12,13,14 this result caused by the highly strained ß-lactam ring in the Amoxicillin and its amide bond break open in the presence of acid and alkaline environment[17,18]. In conclusion, this study showed the maximum stability of Amoxicillin alone and with Potassium clavulanate was experienced at pH values (4,5,6,7,8 and 9), While their degeneration rate was significantly higher at acidic medium (pH=1) and alkaline pH medium (pH= 11, 12 ,13 and 14).

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References

1. Jancel T, Dudas V (2002) Management of uncomplicated urinary tract infections. The Western Jour. Med., 176(1): 51-55.
2. Reading C, Cole M (1977) Clavulanic acid: a beta-lactamase-Inhibiting Beta-lactam from Streptomyces Clavuligerus. Antimicrob. Agents Chemother, 11(5):852-857.
3. Giguere S, Prescott JF, Baggot JD, Walker RD, Dowling PM (2006) Antimicrobial Therapy in Veterinary Medicine. 4th Edition. Blackwell Publishing, Ames, Iowa, 162-165.
4. Wilson R, Anzueto A, Miravitlles M, Arvis P, Faragó G, Haverstock D (2011) A novel study design for antibiotic trials in acute exacerbations of COPD: maestral methodology Int. J. Chron. Obstruct Pulmon Dis., 373-383.
5. Marieb EN, Hoehn K (2010) Human anatomy and physiology. San Francisco: Benjamin Cummings. ISBN 0- 8053-9591.
6. Snyder CH (2003) The Extraordinary Chemistry of Ordinary Things, 4th ed.; John Wiley and Sons: Hoboken, NJ.
7. Waldron K (2007) The Chemistry of Everything. Pearson Prentice Hall: Upper Saddle River, NJ.
8. NCCLS (National Committee for Clinical Laboratory Standards), Approved Standard. NCCLS M38-A. Villanova, PA, USA, 2002.
9. Collee JG, Fraser, AG, Marmion BP, Simmons A (1996) Practical medical microbiology, 14th ed. Churchill Livingston, London.
10. Maysoon SA, Waffa AA, Al-Maaly NM (2017) The inhibition effect of acids and alkalis on the growth of microorganisms. I.J.S.N 8(3): 536-538.
11. Jerzsele A, Nagy G (2009) The stability of amoxicillin trihydrate and potassium clavulanate combination in aqueous solutions. Acta Veterinaria Hungarica, 57(4):485-493.
12. Aki H, Ikeda H, Yukawa M, Iwase Y, Mibu N (2009) Effect of pH on the formation of inclusion complexes between-lactam antibiotics and 2-hydroxypropil-b-Cyclodextrin, in aqueous solution. J Thermal Analy and Calorimet, 95: 421-426.
13. Blaha JM, Knevel AM, Kessler DP, Mincy JW, Hem SL (1976) Kinetic analysis of penicillin degradation in acidic media. J. Pharm. Sci., 65: 1165-1170.
14. Bersanetti PA, Almeida RM, Barboza M, Araujo ML, Hokka CO (2005) Kinetic studies on clavulanic acid degradation. Biochem. Eng. J., 23: 31-36.
15. Sunderland VB, Concannon J , Lovitt H, Ramage M, Tai LH, McDonald C (1986) Stability of aqueous solutions of amoxicillin sodium in the frozen and liquid states. Am J. Hosp. Pharm., 43: 3027-3030.
16. Vahdat L, Sunderland VB (2007) Kinetics of amoxicillin and clavulanate degradation alone and in combination in aqueous solution under frozen conditions. Int. J. Pharm., 342: 95-104.
17. Nagata W (1989) Contribution to the chemistry of ß-lactam antibiotics: 1-oxa nuclear analogs of naturally occurring ß-lactam antibiotics. Pure App. Chem., 61: 325.
18. Hou JP, Poole JW (1971) Lactam antibiotics: their physicochemical properties and biological activities in relation to structure. J. Pharm. Sci., 60: 503.

