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A Comparative Study of Amoxicillin Sensitivity Against *Escherichia coli* Isolates Isolated from Urinary Tract Infections

Sameer Abdul ameer Abid Ali*[1], Ghydaa H. Aljeboury[2]
[1]Deprtement of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
[2]Biotechnology Research Center, AL- Nahrain University, Baghdad, Iraq.

Abstract

The purpose of this study was to investigate the bacterial etiology of urinary tract infections microbiologic properties of *Escherichia coli* isolated from urinary tract infection patients against nine amoxicillin antibiotic. *E.coli* isolates were collected from patients samples suffering from urinary tract infection, based on biochemical tests of *Epi* 20 system .Nine Amoxicillin antibiotics were selected (some vials and other are capsules) which manufactured in different countries were bought from local pharmacies in Baghdad, for the purpose of knowing the inhibitory activity of these antibiotics on *E.coli* one of the main microorganisms to cause urinary tract infection, the antibiotics were prepared in a concentration of 100mg/ml and their activity was tested against the growth of the selected isolates and variations in the results were noted. Antimicrobial susceptibility was tested by the disk diffusion method. Results showed that. *E. coli* reveals variations in the susceptibility to amoxicillin.

Keywords: Urinary Tract Infections, Antibiotic Susceptibility, Amoxicillin, *E.coli.*

دراسة مقارنة حساسية مضاد الاموكسيلين ضد بكتيريا الاشيريشا القولونية المعزولة من التهاب المجاري البولية

سمير عبد الامير عبد علي*[1]، غيداء حسين عبد الرحيم[2]
[1]قسم البايولوجي، كلية العلوم ، جامعة بغداد، بغداد، العراق.
[2]مركز بحوث التقنيات الاحيائية ، جامعة النهرين، بغداد، العراق.

الخلاصة

هدفت الدراسة الحاليه الى دراسة تاثيرالفعالية التثبيطية لمضاد الاموكسيلين المصنع في عدة مناشئ ضد واحدة من اهم المسببات المرضية للمرضى الذين يعانون من التهاب المجاري البولية.جمعت عزلات من بكتيريا *الاشيريشا القولونية* لمرضى يعانون من التهاب المجاري البولية وعزلت البكتيريا وشخصت العزلات اعتمادا على الاختبارات الكيوحيوية لفحوصات العدة التشخيصية الابي 20 الخاصة بالعائلة البكتيرية المعوية. اختيرت تسعة مناشئ لتصنيع المضاد اموكسيلين في عدة مدن وقد تم شراؤه من الصيدليات المحلية في بغداد،ولمعرفةالتاثيرالتثبيطي لهذه المضادات على بكتريا القولون المسبب التهاب المجاري البوليه، حضر المضاد الحيوي اموكسيلين بتركيز 100 ملغم / مل لغرض الدراسه.اختبرت الفعالية التضادية باستخدام طريقة الانتشار في الحفر للوسط حيث اظهرت النتائج تغاير في حساسية البكتيريا تجاه مضاد الاموكسيلين.

*Email: sameer_alash@yahoo.com

Introduction

Urinary tract infections (UTIs) are one of the most common infectious diseases [1]. They may be symptomatic or asymptomatic, and either type of infection can result in serious sequelae if not appropriately treated [2]. Although different causative agents can be responsible for UTIs, bacteria are the major cause being responsible for more than 95% of UTI cases [3].In this context, *E. coli* is the most prevalent organism and is solely responsible for the majority of these infections. An accurate and prompt diagnosis is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract [4].The antibacterial mechanism of action of amoxicillin consists of the inhibition of the biochemical processes of synthesis of the bacterial wall [5]. This is done by means of selective and irreversible blockage of different enzymes that are involved in such processes, principally transpeptidases, endopeptidases and carboxypeptidases . Treatment of UTI is often started empirically. UTIs are often treated with different broad-spectrum antibiotics when one with a narrow spectrum of activity may be appropriate because of concerns about infection with resistant organisms, and antimicrobial susceptibility testing of the urinary pathogens constitutes the basis for antibiotic therapy [6]. However, in view of the increasing bacterial resistance, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy [7, 8]. The inadequate formation of the bacterial wall, in susceptible species, produces an osmotic imbalance that especially affects bacteria in their growth phase (during which the processes of the synthesis of bacterial walls are very important), which leads in the end to the lysis of the bacterial cell [8, 9]. The present study aims at analyzing the infectious epidemiology of UTIs in a general local hospital located in Baghdad. In addition, it examines the susceptibility of *E. coli* against nine amoxicillin antibiotic.

Materials and Methods:

Antibiotic:

Amoxicillin 500mg/5ml (from different countries) were purchased from local pharmacies in Baghdad, Iraq:

1. Kopran pharmaceuticals (India) - Capsule
2. JPI (Saudi Arabia)- Capsule
3. ACAI (Iraq) - Capsule
4. Global pharma (U.A.E) - Capsule
5. TROGE (Germany)- Vial
6. Kontam pharmaceuticals (China) - Capsule
7. PAN (France)- Vial
8. Athlone laboratories (Ireland) - Capsule
9. Neopharma (Abu Dhabi) – Capsule

Methods:Isolation and Identification of Organisms:

A total samples were taken from patients suffering from urinary tract infections inoculated on blood agar as well as MacConckey agar and incubated at 37°C for 24 hr as positive cases, and for 48 hr in negative cases[10]. A specimen was considered positive for UTI in the light of the number of yielded colonies ($\geq 10^5$ cfu/mL). Bacterial identification was based on standard culture and biochemical characteristics of isolates based on detecting API 20 E for enterobacterecieae family [11, 12].Gram-negative bacteria were identified by standard biochemical tests, Gram-positive microorganisms were identified with the corresponding recommended laboratory tests [13].

Susceptibility Testing:

Antimicrobial susceptibility of *E. coli* was tested by the disk diffusion method according to the method recommendation, by using the Mueller-Hinton agar [14]. Antimicrobial agents of amoxicillin was tested against bacterial growth of *E.coli* .The zone of inhibition was measured in mm by the aid of a metric ruler.

Antibiotic preparation for sensitivity test:

1. Distilled water was sterilized by autoclave for 20 min
2. The antibiotic 500 mg (one capsule/whole vial) dissolved in 5 ml of sterile D.W by a sterile syringe to get a conc. of 100mg/ml. Note: the capsule powder was transferred to plastic tube with screw cap then dissolved while the vials were dissolved in their original tubes.
3. The filter paper was cut into small circles of 5 mm with a perforator and then transferred to petri dishes.
4. The cut filter paper was submerged with the antibiotic solution.

5. Then the petri dishes were placed with the lids open to the middle in the incubator at 35º C for 24 hrs. to let the filter paper dry but keep a bit of moisture.
6. Then they were kept at 4º C until use.
7. The plates were inoculated by dipping a sterile swab into the inoculums. The excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.
8. The swab was rubbed all over the surface of the medium three times rotating the plate through an angle of 60 after each application. Finally, the swab was passed around the edge of agar surface.
9. The inoculums were left to dry for a few minutes at room temperature with the lid closed.
10. By using a sterile forceps, the selected amoxicillin filter paper were placed on the inoculated plate (each plate with 3 filter papers). Discs should be warmed to room temperature, and then dispensed on the agar surface; they should gently press down with forceps, the plates were labeled and incubated for 18-24 hrs at 37ºC.

Results and Discussion:

As expected, *E. coli* was the most frequent isolate throughout the ten years . It was followed by *Klebsiella pneumonia* and the next most frequently isolated bacteria were *Proteus* sp., *Pseudomonas aeruginosa* , *Enterococcus* sp., and *Streptococcus agalactiae* , in this study, E. coli accounted for approximately 61% of all clinically significant urinary isolates and 76.8% of all *Enterobacteriaceae* . This is consistent with the findings of previous studies in which *E. coli* was the predominant pathogen isolated from patients with UTIs [14]. *E. coli* isolates from urinary infections show a similar pattern of susceptibility to those isolated from all body site infections although with a more enhanced susceptibility percentages. This study shows the distribution of microbial species and antibiotic susceptibility patterns of *E. coli* isolated from patients with UTIs .Results showed that all of the amoxicillin types which were used in this research expressed an antibacterial activity in variable degrees toward the different strains of *E.coli*, except for *E.coli* (c), which was sensitive only to amoxicillin (8 &9) while it had 100% resistance to (1, 2, 3, 4, 5, 6, 7).Amoxicillin type (1) till (7) inhibited other isolates of *E.coli*, so it can't be regarded as an inactive or an adulterated antibiotic , while *E.coli* (e) was 100% resistant for amoxicillin (4, 5, 8, and 9) while it was sensitive to the others. Also the results showed that *E.coli* (d) was 100% resistant for amoxicillin (1) while it was sensitive to the others .These results indicate the capability of some types of *E.coli* to resist the antibacterial effect of some amoxicillin, because of having its own mechanisms, which confer the bacterial resistance [15] .The variability in the amoxicillin effects may be due to the differences in the industrial techniques and chemical composition .All the nine types of amoxicillin tested couldn't accomplish complete inhibitory action on all the isolates of *E.coli* in about 100%. Amoxicillin (2,3,6,7,8,9) have recorded higher antibacterial effects compared with amoxicillin (1,4,5) which were less effective .The rates of susceptibility (and subsequently of non susceptibility including both resistant and intermediately resistant strains) of *E. coli* isolates to antibiotics which are commonly used to treat *Escherichia* infections. The lowest rate of susceptibility was manifested against amoxicillin (between 0-0.5mm) followed by moderates (between 0.5-2mm), whereas high susceptibility was observed with (0.5-2.8mm) Figure-A. Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. The resistance to the antimicrobials has increased over the years, resistance rates vary from country to country [16]. In this study, *E. coli* accounted for approximately 61% of all clinically significant urinary isolates and 76.8% of all *Enterobacteriaceae* . This is consistent with the findings of previous studies in which *E. coli* was the predominant pathogen isolated from patients with UTIs [17, 18]. *E. coli* isolates from urinary infections show a similar pattern of susceptibility to those isolated from all body site infections although with a more enhanced susceptibility percentages. After incubation of isolates the diameters of the complete zone of inhibition were noted and measured in millimeters by the aid of a metric ruler. The diameter of inhibition zone for individual amoxicillin antibiotic disc was translated in terms of charts to compare between the antibiotic (Amoxicillin) effective from different countries [19, 20]. All figures below illustrate the variation in amoxicillin activity among *E.colii* solates.

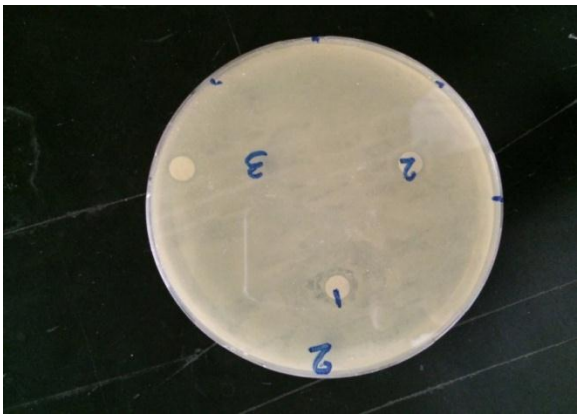

Figure 1- Inhibitory effect of 1: Kopran Indian Amoxicillin; 2: JPI Saudi Arabia Amoxicillin; 3: ACAI Iraqi Amoxicillin on isolate *E.coli*: 2.

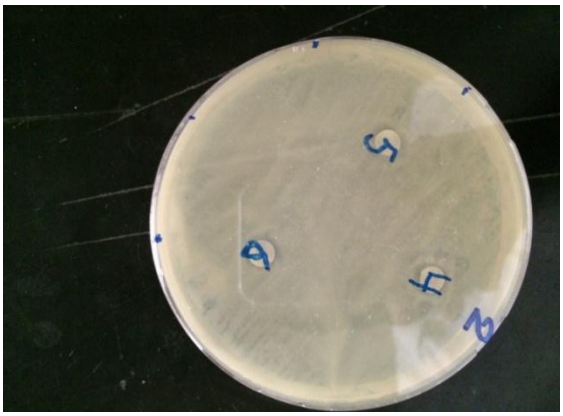

Figure 2- Inhibitory Effect of 4: Global pharma U.A.E Amoxicillin; 5: TROGE German Amoxicillin; 6: kontam Chinese Amoxicillin on isolate *E.coli*: 2.

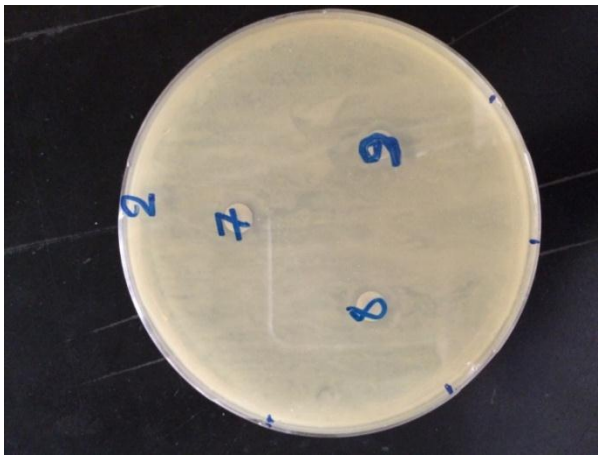

Figure 3 - Inhibitory Effect of 7: PAN French Amoxicillin; 8: Athole laboratory Irish Amoxicillin;9: Neopharma Abu Dhabi Amoxicillin on isolate *E.coli*: 2.

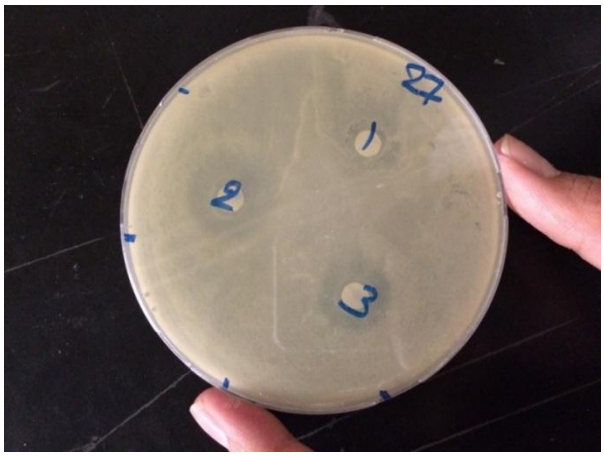

Figure 4 - Inhibitory Effect of 1: Kopran Indian Amoxicillin; 2: JPI Saudi Arabia Amoxicillin; 3: ACAI Iraqi Amoxicillin on isolate *E.coli*: 27.

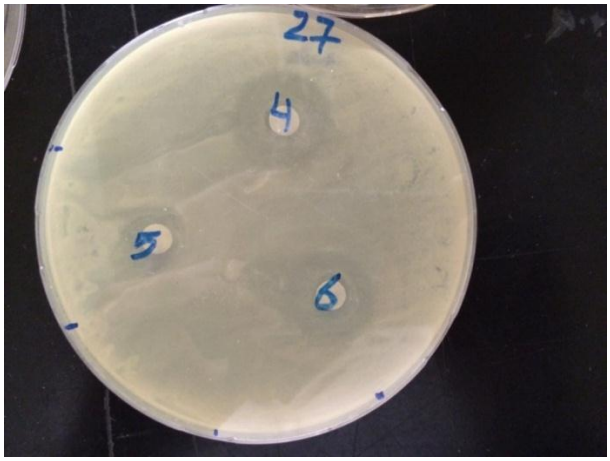

Figure 5 - *Inhibitory Effect of 4: Global pharma U.A.E Amoxicillin; 5: TROGE German Amoxicillin; 6: kontam Chinese Amoxicillin on isolate E.coli: 27.*

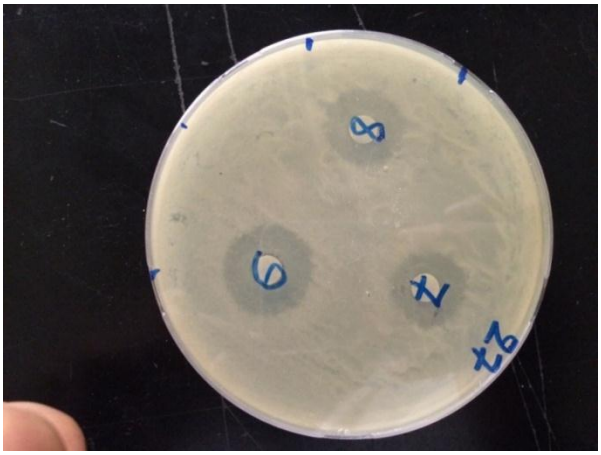

Figure 6- Inhibitory Effect of 7: PAN French Amoxicillin; 8: Athole laboratory Irish Amoxicillin; 9: Neopharma Abu Dhabi Amoxicillin on isolate *E.coli*: 27.

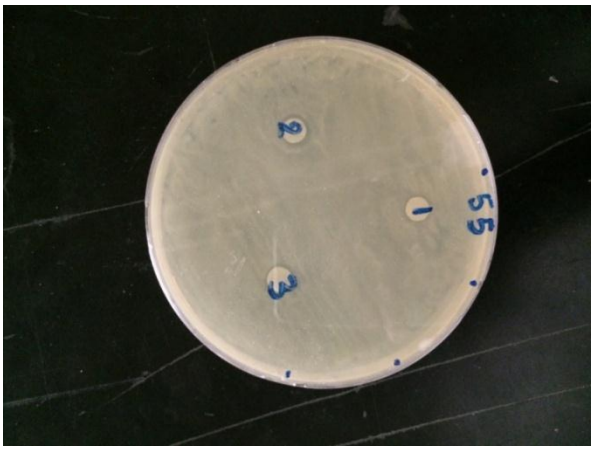

Figure 7 - Inhibitory Effect of 1: Kopran Indian Amoxicillin; 2: JPI Saudi Arabia Amoxicillin; 3: ACAI Iraqi Amoxicillin on isolate *E.coli*: 55.

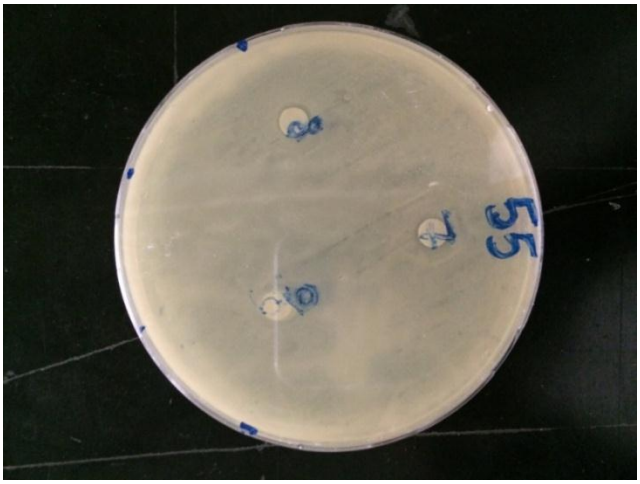

Figure 8- *Inhibitory Effect of 4: Global pharma U.A.E Amoxicillin; 5: TROGE German Amoxicillin; 6: kontam Chinese Amoxicillin on isolate E.coli:55.*

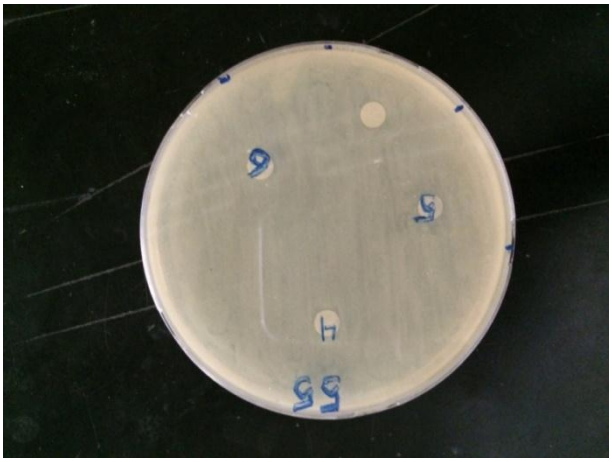

Figure 9- Inhibitory Effect of 7: PAN French Amoxicillin; 8: Athole laboratory Irish Amoxicillin; 9: Neopharma Abu Dhabi Amoxicillin on isolate *E.coli*: 55.

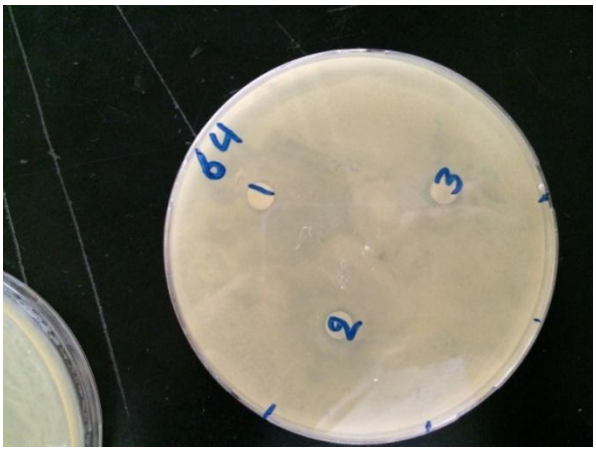

Figure 10- Inhibitory Effect of 1: Kopran Indian Amoxicillin; 2: JPI Saudi Arabia Amoxicillin; 3: ACAI Iraqi Amoxicillin on isolate *E.coli*: 64.

Figure 11- Inhibitory Effect of 4: Global pharma U.A.E Amoxicillin; 5: TROGE German Amoxicillin; 6: kontam Chinese Amoxicillin on isolate *E.coli*: 64.

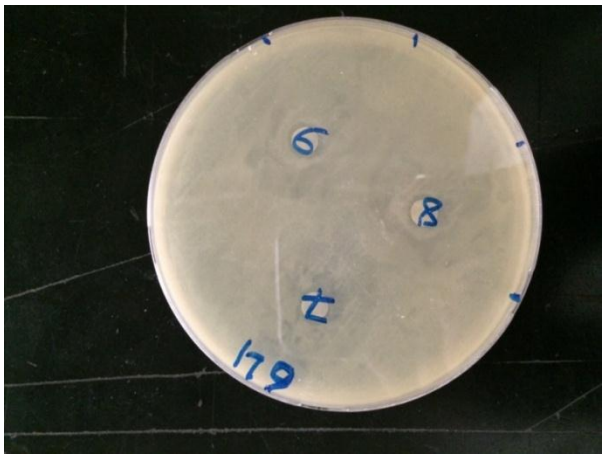

Figure 12- Inhibitory Effect of 7: PAN French Amoxicillin; 8: Athole laboratory Irish Amoxicillin; 9: Neopharma Abu Dhabi Amoxicillin on isolate *E.coli*: 64.

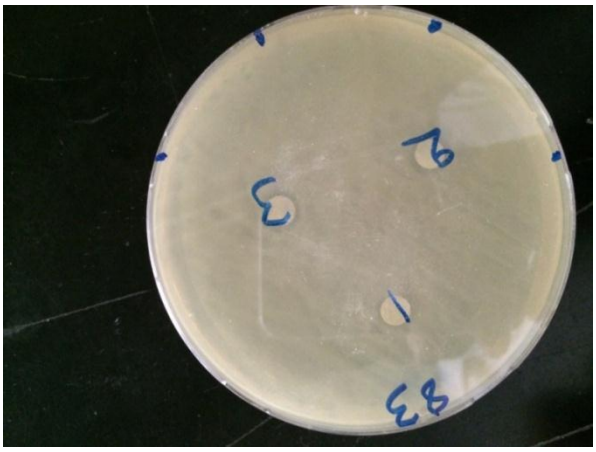

Figure 13-Inhibitory Effect of 1: Kopran Indian Amoxicillin; 2: JPI Saudi Arabia Amoxicillin; 3: ACAI Iraqi Amoxicillin on isolate *E.coli*: 83.

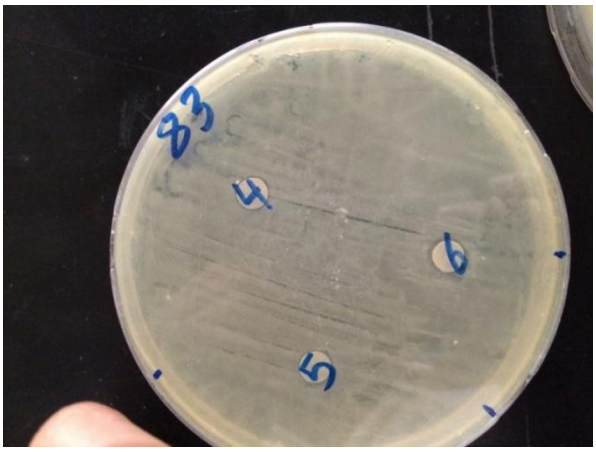

Figure 14- Inhibitory Effect of 4: Global pharma U.A.E Amoxicillin; 5: TROGE German Amoxicillin; 6: kontam Chinese Amoxicillin on isolate *E.coli*: 83.

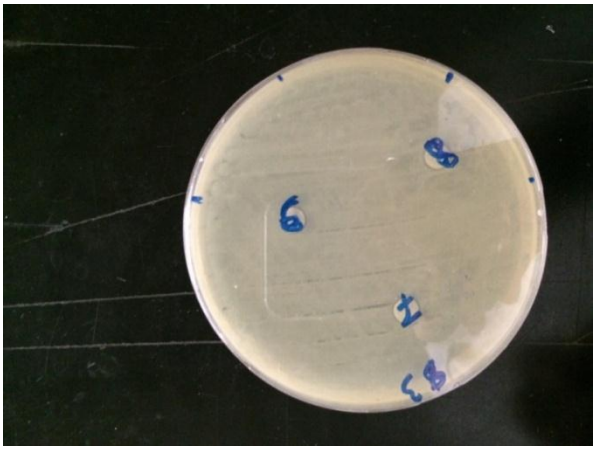

Figure15- Inhibitory Effect of 7: PAN French Amoxicillin; 8: Athole laboratory Irish Amoxicillin; 9: Neopharma Abu Dhabi Amoxicillin on isolate *E.coli*: 83.

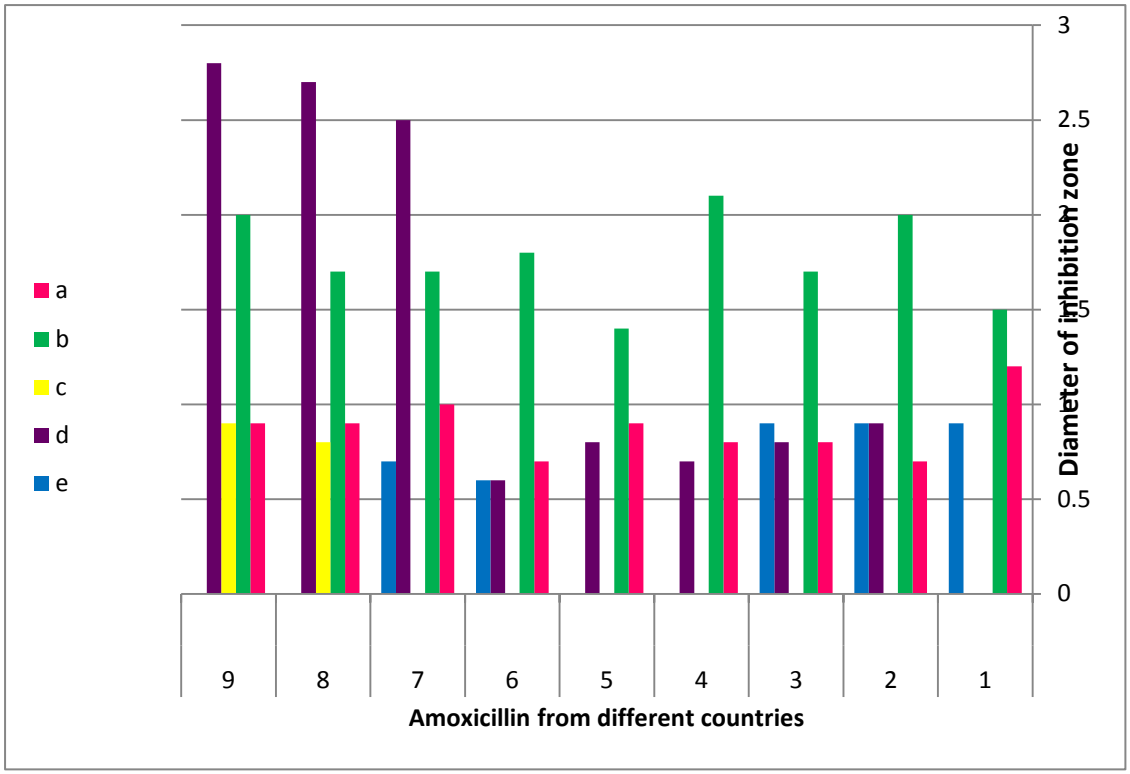

Figure- A- The antibacterial activity of amoxicillin from different origin on *E.coli* isolates.

Note:

E.coli isolate *2* **a**
E.coli isolate 27 **b**
E.coli isolate 55 **c**
E.coli isolate 64 **d**
E.coli isolate 83 **e**

Conclusion

- Amoxicillin from different countries did not show the same inhibitory activity due to different mechanisms used in the manufacturing of the different amoxicillin.
- *E.coli* showed different degrees of resistance to amoxicillin due to different resistance mechanisms used by the different strains of *E.coli* like the efflux pump, target substrate configuration, enzyme production and modification and degradation.
- These results give as the indication in depending the antibiotic sensitivity tests for *E.coli* isolated from urinary tract infection in order to determine the actual source of antibiotics that has the highest antibacterial activity, in this case, the treatment would not be randomly chosen and the treatment will not fail.
- Also this research indicated that all types of amoxicillin imported to Iraq have a variable antibacterial effect(source Abu Dhabi gave best results) and other times does not work completely depending on the bacterial resistance.

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