

Effect of Different Brands of Ceftriaxone on Various Strains of Bacteria by Measuring Zone of Inhibition

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تأثير أصناف مختلفة من السفترياكسون على عدة أنواع من البكتيريا بقياس منطقة التثبيط

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Abstract

In this study, the antibiotic ceftriaxone from three different brands was tested for its effectiveness against strains of bacteria like *Streptococcus pyogenes*, *Staphylococcus aerus*, and *Klebsiella pneumonia*. The effectiveness was estimated by measuring the zone of inhibition and compared with a standard disc (BD BBL Sensi-Disc 2004/09) from an American company-general lab. The diameters of the inhibitory zones from all the brands were found to be well within the range of 20 to 33 mm \pm 0.5.

Keywords: Ceftriaxone, Zone of Inhibition.

الملخص

السفترياكسون مضاد حيوي واسع المجال من الجيل الثالث من السيفالوسبورين. في هذه الدراسة أخذت ثلاثة أصناف منتجة بواسطة ثلاثة شركات مختلفة (تونسية وإسبانية وتركية) وتم اختبار فاعليتها على ثلاثة أنواع من البكتيريا (ستربتوكوكس بيوجينز وستافيلوكوكس اوريس وكلبسيليا نيمونيا) وتم قياس قطر منطقة تثبيط النمو لمعرفة تأثير كل صنف وتمت مقارنتها مع القرص العياري BDBBL-disc 2004/09 من شركة المختبرات الأمريكية وقد وجد أن جميع الأصناف لها فاعلية جيدة ضد الأنواع الثلاثة من البكتيريا المذكورة وكان قطر منطقة التثبيط في مدى من 20 إلى 33 مم.

الكلمات الدالة: السفترياكسون، منطقة التثبيط.

1. Introduction

Ceftriaxone is a new semi-synthetic, broad spectrum, third-generation, cephalosporin that needs to be administered once-daily. It is a β -lactamase-resistant cephalosporin and is active against gram-positive and most gram-negative bacteria. The mechanism of action of Ceftriaxone, like any other cephalosporin, is by interfering cell walls synthesis in bacterial cells (Harvey, 2009).

The drug has better penetration into the cerebrospinal fluid and is useful in the treatment of bacterial meningitis. The dose of ceftriaxone is 50 mg/kg per day in neonates and 100 mg/kg per day in older infants. It displaces bilirubin from albumin binding sites and increases the unconjugated free bilirubin in plasma. It should only be given with great caution in infants with high unconjugated plasma bilirubin level (Pacifci, 2010).

Ceftriaxone has a longer half-life than other cephalosporin; the plasma half-life of ceftriaxone is 15 hrs at birth and 7 hrs over 2-4 weeks (Katzung *et al.*, 2012). Bacteria can be broadly classified according gram staining of their cell walls as gram positive bacteria (cocci) like *staphylococcus* and *streptococcus* and gram negative bacteria (bacilli, rods) like *Escherichia coli* and *Klebsiella* (Willey *et al.*, 2016).

In the present aims to evaluate the efficacy of different brands of ceftriaxone against strains of bacteria like *staphylococcus aurius*, *streptococcus pyogens* and *Klebsiella*.

2. Material and Methods

2.1. Study design

In this experiment, the effectiveness of three different brands of Ceftriaxone on three strains of bacteria was determined by measuring and comparing their zones of inhibitions with control (Cappuccino and Sherman, 2011). The bacterial samples (*Streptococcus pyogens* type B, *Staphylococcus* and *Klebsiella pneumonia*) were isolated from sample analysis laboratory in Zliten Teaching Hospital between May to August 2018 (Cappuccino and Sherman, 2011; and Hsueh *et al.*, 2010).

The bacterial strains were identified and their testing of susceptibility was done with BD Phoenix™ and API method. Furthermore, three different brands of Ceftriaxone injection dosage form having strength of 1 g/ml were used. The three brands were Cefaxone (Les laboratories, Unimed, Tunisia), Decefin (Devaholding A.S., Turkey) and Cefrixone (Normon, Spain).

The stock solution of antibiotics was prepared in the following manner. One ampule was diluted to 1000 ml. The formula

$$W = \frac{1000}{P} \times V \times C \quad \dots\dots\dots (1)$$

Where *P* is potency of antibiotics, *V* is volume (ml) required; *C* is final concentration of solution (mg/ml), and *W* is weight of the antimicrobial taken (mg). The Mueller-Hinton agar was used for culturing *S. Pyogens type B*, *Staphylococcus* and *Klebsiella pneumonia* (Hsueh *et al.*, 2010; and Hudzicki, 2009).

2. 2. Microbiological methods

The Mueller-Hinton agar media, 8 in number were prepared for inoculation and culture of *S. Pyogens type B*, *Staphylococcus* and *Klebsiella pneumonia*. The agar plates were labelled and marked for standard, test samples and control (Cappuccino and Sherman, 2011; and Hsueh *et al.*, 2010).

Under aseptic conditions, inoculate one plate with *Klebsiella pneumonia* broth culture using a sterile swab. Thoroughly swab the surface of the plate to cover the entire surface turn the plate to approximately 60° and repeat the previous step (Cappuccino and Sherman, 2011; and Hudzicki, 2009)

The standard disc was put into the surface of agar using sterile technique. Two holes were bored with help of sterile borer having diameter of 10 mm and test samples of the antibiotic were poured in each hole. This procedure was similar for the plates containing *S. Pyrogens type B*, *Staphylococcus* and *Klebsiella pneumonia* culture. All the plates were left for 5 mins. in order to dry completely. The plates were incubated at 37 °C in German incubator for 48 hrs. The diameters for the zone of inhibitions were measured by grade ruler and results were compared with that of the standard disc (Hsueh *et al.*, 2010 and British Medical Association, and Royal Pharmaceutical Society of Great Britain, 2009)

2.3. Statistical analysis

The categorical variables between the plates were analyzed using the excel program of Microsoft 2016 to measure average values of inhibitory zones of all samples, means and standard deviations were compared with the standard.

3. Results and Discussion

The range of inhibitory zones was within >20 mm. The result of control was 21.33±0.1.15 mm which indicated that it is effective against *Staphylococcus aureus*. on the other hand. Cefaxone (Les laboratories, Unimed, Tunisia) gave highest effect as compared to control. Its zone of inhibition diameter was found to be 25.66±1.15 mm. The least effective brand on inhibition of *Staphylococcus aureus* was Ceftriaxone (Normon, Spain) having a diameter of 20±1 mm (as mentioned in Table 1, and Figure 1).

Table 1. Zone of inhibition of three brands of ceftriaxone against *Staphylococcus aerus*

Dose of Ceftriaxone 30 µg	<i>Staphylococcus aerus</i>			
	Exp-01	Exp-02	Exp-03	Mean± SD
Control	22	22	20	21.33±1.15
Turkey	27	25	23	25± 2
Tunisia	25	27	25	25.66±1.15
Spain	21	19	20	20±1

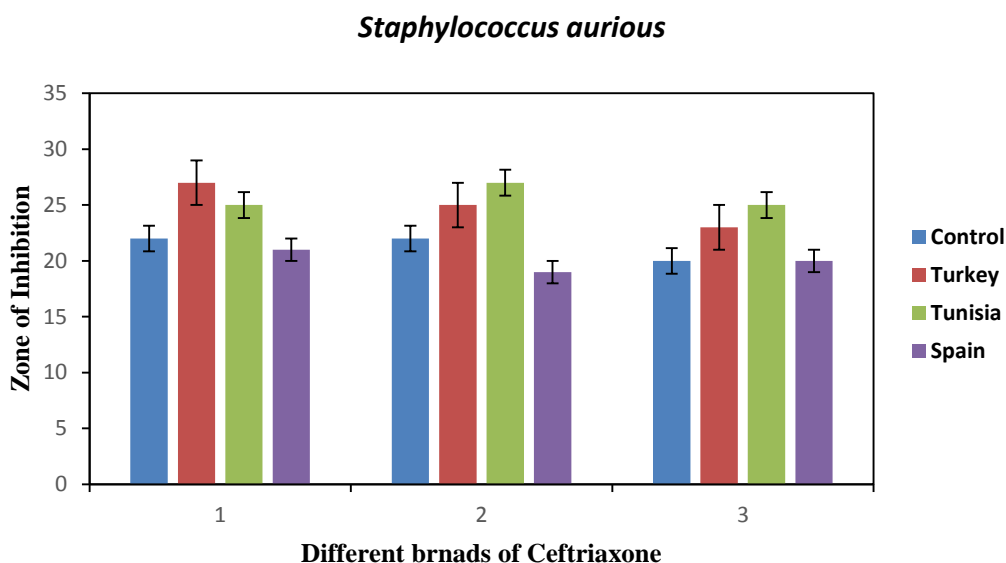


Figure 1. Effect of three Ceftriaxone brands against *Staphylococcus aurious*

In case of *Streptococcus* strain, the mean inhibitory zone control was 24.33 ± 1.15 mm as compared to control. The diameters of the inhibitory zones of ceftriaxone for Cefaxone (Les laboratories, Unimed, Tunisia) and Ceftrixone (Normon, Spain) were similar, 27.66 ± 0.57 mm, whereas Decefin (Devaholding A.S., Turkey) gave 26.66 ± 0.57 mm. All these results showed inhibitory diameters were well within the required range for *Streptococcus pyogens* types B (as mentioned in Table 2, and Figure 2).

Table 2. Zone of inhibition of three brands of ceftriaxone against *Streptococcus pyogens*

Dose of Ceftriaxone 30 µg	<i>Streptococcus pyogens</i>			
	Exp-01	Exp-02	Exp-03	Mean±SD
Control	25	23	25	24.33±1.15
Turkey	27	26	27	26.66±0.57
Tunisia	27	28	28	27.66±0.57
Spain	27	28	28	27.66±0.57

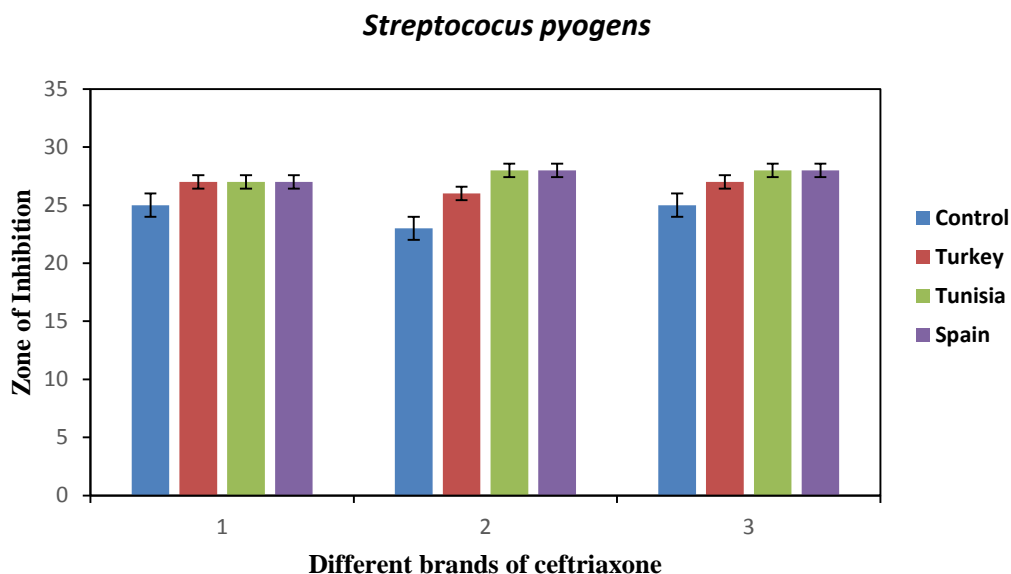


Figure 2. Effect of three Ceftriaxone brands against *Streptococcus pyogens*

For the bacteria *Klebsillea Pneumonia*, the mean diameter of inhibitory zone for control was 25 ± 3.0 mm. The diameter of inhibitory zone for Decefin (Devaholding A.S., Turkey) was 33 mm. The mean diameter of inhibitory zone for Cefaxone (Les laboratories, Unimed, Tunisia) was 29 ± 6.55 mm. The mean diameter for the zone of inhibition Ceftriaxone (Normon, Spain) was 31.6 ± 1.52 mm (as mentioned in Table 3, and Figure 3).

Table 3. Zone of inhibition of three brands of ceftriaxone against *Klebsiella pneumoniae*

Dose of Ceftriaxone 30 μ g	<i>Klebsiella pneumoniae</i>			
	Exp-01	Exp-02	Exp-03	Mean \pm S.D
Control	25	28	22	25 ± 3
Turkey	33	33	33	33
Tunisia	30	22	35	29 ± 6.55
Spain	30	32	33	31.66 ± 1.52

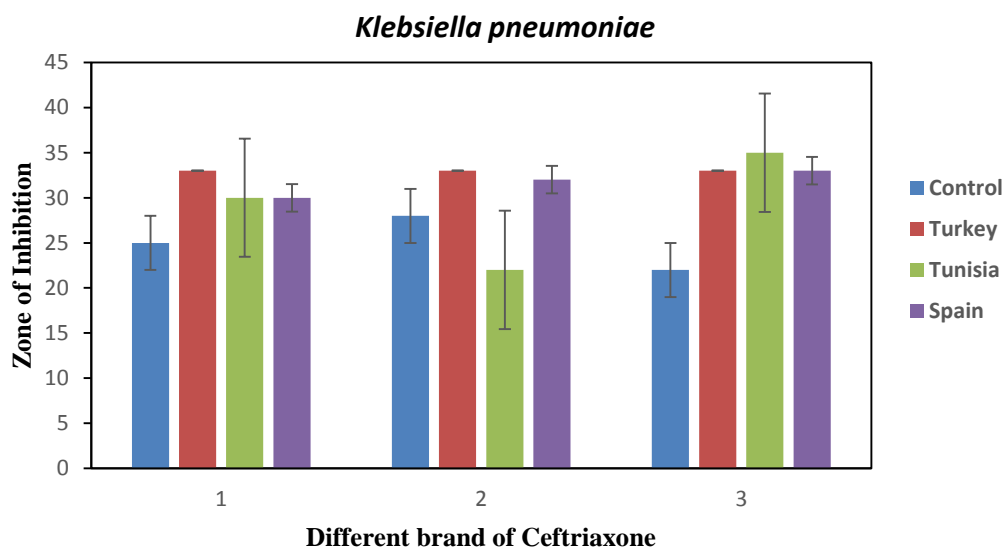


Figure 3. Effect of three ceftriaxone brands against *Klebsiella pneumonia*

The result showed that there is no significant variation between the brands in term of content and potency. The results with both the strains of bacteria were same in response to antibiotics treatment. Although the mean of diameters of each brand were different, but they were within the pharmacopeia limit

The past studies, reported these studies without exactly measuring the zone of inhibition. In present study sensitivity, susceptibility of bacteria investigated and resistance of the bacteria used bacteria checked (Willey *et al.*, 2016).

4. Conclusion

It was concluded that the used brands had different diameters of inhibitory zones due to storage or other physicochemical reasons. But Cefaxone (Les laboratories, Unimed, Tunisia), Decefin (Devaholding A.S., Turkey) and Ceftriaxone (Normon, Spain) showed similar bactericidal effect on *Staphylococcus pyogens type B*. Therefore, this type of study can be incorporated on other brands of different antibiotics as criteria to accept or reject them and to improve their efficacy.

5. Recommendations

The main recommend through this study the Libyan Medical Labs who are in responsibility to take action to minimize or prevent the any drug adulteration and checking the quality of all drugs. The other brands needed to be tested which is not taken by Libyan health organization.

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