

## Evaluation the efficacy of three brands of Ceftriaxone against various strains of bacteria by measuring zone of inhibition

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### Abstract

Ceftriaxone (Rocephin) is wide spectrum cephalosporin that has been used for decades for combating infections caused by broad array of pathogens. In this study, the antibiotic ceftriaxone from three different brands was tested for its effectiveness against strains of bacteria like *Streptococcus pyrogenes*, *Staphylococcus aureus* and *Klebsiella pneumonia*. The effectiveness of ceftriaxone was estimated by measuring zone of inhibition and compared with standard disc (BD BBL Sensi-Disc 2004/09). The diameters of the inhibitory zones for all brands were ranged from 20 to 33mm±0.5. The present study showed that there is no significant variation amongst the different brands of ceftriaxone in term of their antibacterial effectiveness.

**Keywords:** Ceftriaxone, Zone of Inhibition, antibacterial efficacy

<https://doi.org/10.54361/ljmr.v14i2.03>

### 1-Introduction

Ceftriaxone (Beta-lactam antibiotics) is a broad spectrum third generation of cephalosporin. [1]. It is highly effective against Gram negative as well as Gram positive organisms. Ceftriaxone is

unique because of its prolonged serum half-life, which permits once- or twice-daily dosing of this member of Cephalosporin family. [2]. 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 [3].

Ceftriaxone has a longer half-life than other cephalosporin; the plasma half-life of ceftriaxone is 15

## 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 [5].

The bacterial samples (*Streptococcus pyrogenes* type B, *Staphylococcus aureus* and *Klebsiella pneumonia*) were isolated from sample analysis laboratory in Zliten Teaching Hospital between May to August

hours at birth and 7 hours over 2-4 weeks. [4].

It has high potency against all the *Enterobacteriaceae*, *Haemophilus influenzae*, the *Nisseria* and most Gram-positive cocci except *Enterococcal spp.* [5].

This study aimed to evaluate the efficacy of different brands of ceftriaxone locally available against strains of bacteria like *staphylococcus aureus*, *streptococcus pyogenes* and *Klebsiella*.

2018 following the standard procedure [5, 6].

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 1g/ml were used. The three brands were Ceftriaxone (B), (A) and Ceftriaxone (C).

The stock solution of antibiotics was prepared in the following

manner. One ampule was diluted to 1000 ml. The formula

$$W = \frac{1000}{P} \times V \times C$$

Where P is potency of antibiotics, V is volume (ml) required; C is

## 2, 2. Microbiological methods

The Mueller-Hinton agar media, 8 in number were prepared for inoculation and culture of *S. Pyrogens type B*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The agar plates were labelled and marked for standard, test samples and control [5, 7].

Under aseptic conditions, inoculate one plate with *Klebsiella pneumoniae* 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 [5, 7].

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

final concentration of solution (mg/ml) and W is weight of the antimicrobial taken (mg). The Mueller-Hinton agar was used for culturing *S. Pyrogens type B*, *Staphylococcus* and *Klebsiella pneumoniae* [6, 7].

poured in each hole. This procedure was similar for the plates containing *S. Pyrogens type B*, *Staphylococcus* and *Klebsiella pneumoniae* culture. All the plates were left for 5 minutes in order to dry completely. The plates were incubated at 37°C in German incubator for 48 hours. The diameters for the zone of inhibitions were measured by grade ruler and results were compared with that of the standard disc [6, 7, and 8].

## 2, 3. Statistical analysis

The categorical variables between the plates were analysed 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.

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### 3. Result and Discussion

The results of treated samples were as following; the highest inhibitory zone was found in bacterial culture treated with brand B ceftriaxone ( $25.66 \pm 1.15$ ), however, the sizes of inhibitory zone in brand A and brand C ceftriaxone were found  $25 \pm 2$ mm and  $20 \pm 1$ mm respectively (Table 1, Figure 1). On the other hand, the size of inhibitory zone in control samples was  $25.66 \pm 1.15$  mm which indicated that it is more effective against *Staphylococcus aureus*.

In case of *Streptococcus* strain, the mean inhibitory zone was  $24.33 \pm 1.15$ mm as according to the control. The diameters of the inhibitory zones of ceftriaxone for Ceftriaxone (B) and Ceftriaxone (C) were similar,  $27.66 \pm 0.57$ mm, whereas (A) gave  $26.66 \pm 0.57$ mm. All these results showed inhibitory diameters were adequately within the required range for *Streptococcus pyrogens* types B (table 2, figure 2).

For the bacteria *Klebsiella Pneumonia*, the mean diameter of inhibitory zone for control was  $25 \pm 3.0$ mm. Furthermore, the diameter

of inhibitory zone for (A) was 33mm. The mean diameter of inhibitory zone for Ceftriaxone (B) was  $29 \pm 6.55$ mm. The mean diameter for the zone of inhibition Ceftriaxone (C) was  $31.6 \pm 1.52$  mm. (Table 3, figure 3).

According to (BNF 57, 2009), Ceftriaxone was showed the following results, *S. aureus* was 96.1% of the isolates inhibited by ceftriaxone. While, *E. coli* (95%), *P. aeruginosa* (92.7%), *K. pneumonia* (89.4%) and *S. typhi* (87.2%). [9]

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.

Minimum inhibitory concentration (MIC) for the three ceftriaxone brands was ranged from  $<0.25$  to  $>256$  mg/L. The MIC for both A and C ceftriaxone were reliable for

all isolates. Sequentially, MIC for brand B was higher by at least double concentration, suggesting this brand had the lowest in-vitro activity against isolates tested. [10]

In this study, *Staphylococcus aureus*, which represents 19% of

#### 4. Conclusion

This aspect of the research suggested that, the used brands had different diameters of inhibitory zones and this may be due to the storage condition or other physicochemical reasons. Alternatively, in this study, Ceftriaxone(B), (A) and

the total isolates tested, showed 23.4% (11/47) and 34% (16/47) resistance to ceftriaxone and ceftazidime, respectively.

Correspondingly,

*Klebsiella pneumoniae* showed 46.1% (12/26) resistance to ceftriaxone. [11]

(C), showed similar bactericidal effect on *Staphylococcus pyogens* (type B). Therefore, this type of study can be incorporated on other brands of different antibiotic classes as criteria to evaluate them and to improve their efficacy

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
Control	27	25	23	25± 2
(A)	25	27	25	25.66±1.15
(B)	21	19	20	20±1

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

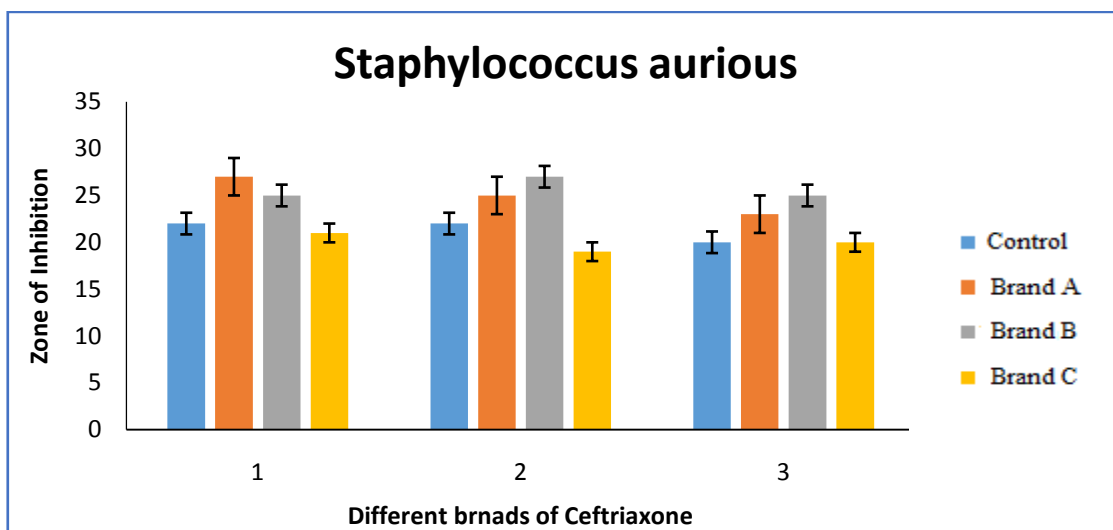


Figure 1, Effect of three ceftriaxone brands against staph.aurious

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
(A)	27	26	27	26.66±0.57
(B)	27	28	28	27.66±0.57
(C)	27	28	28	27.66±0.57

Table 2, zone of inhibition of three brands of ceftriaxone against *Streptococcus pyogens*

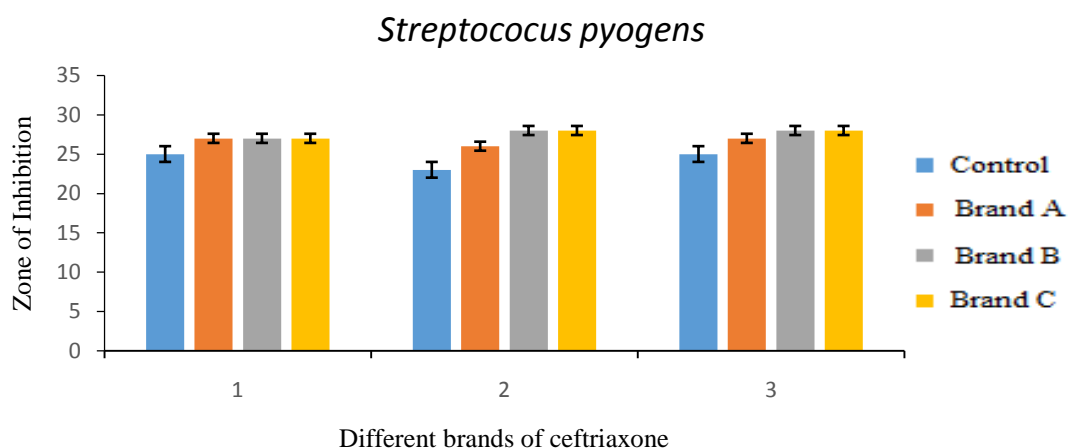


Figure 2, Effect of three ceftriaxone brands against streptococcus pyogens

Dose of Ceftriaxone 30 µg	<i>Klebsiellapneumoniae</i>			
	Exp-01	Exp-02	Exp-03	Mean ±S.D
Control	25	28	22	25±3
(A)	33	33	33	33
(B)	30	22	35	29±6.55
(C)	30	32	33	31.66±1.52

Table 3, zone of inhibition of three brands of ceftriaxone against *Klebsiellapneumoniae*

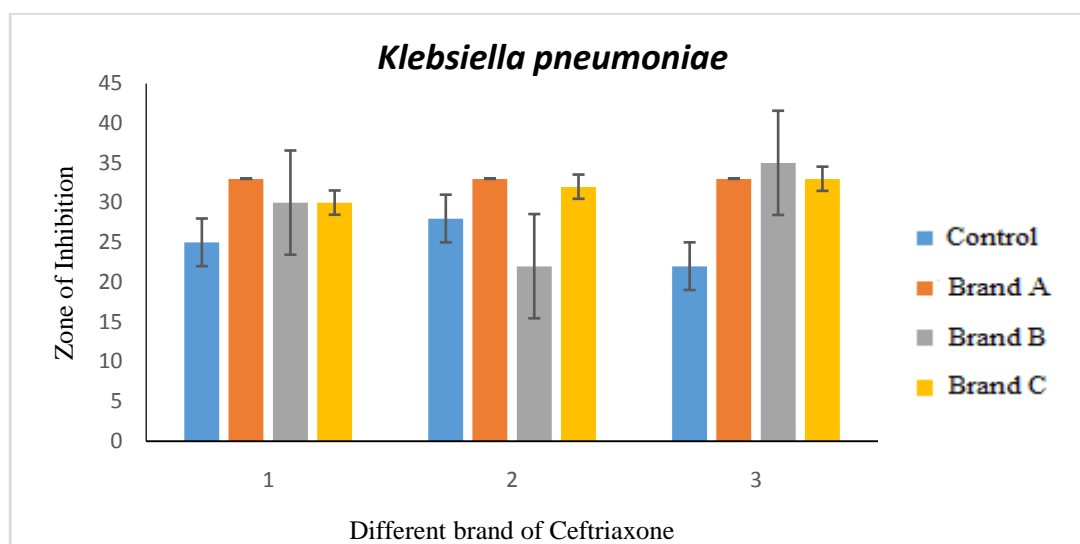


Figure 3 ,Effect of three ceftriaxone brands against *klebsilla pneumonia*

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