

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

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

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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 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 pyogens* and *Klebsiella*.

Material and methods

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 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 final concentration of solution (mg/ ml) and W is weight of the antimicrobial taken (mg). The Mueller-Hinton agar was used for culturing *S. Pyogenes type B*, *Staphylococcus* and *Klebsiella pneumonia*.^{6,7}

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 pneumonia*. The agar plates were labelled and marked for standard, test samples and control.^{5,7} 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.^{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 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 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.⁶⁻⁸

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.

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±1.15), however, the sizes of inhibitory zone in brand A and brand C ceftriaxone were found 25±2mm and 20±1mm respectively (Table 1, Figure 1). On the other hand, the size of inhibitory zone in control samples was 25.66±1.15mm which indicated that it is more effective against *Staphylococcus aureus*.

Table 1 Zone of inhibition of three brands of ceftriaxone against *Staphylococcus aureus*

Dose of Ceftriaxone 30µg	<i>Staphylococcus aureus</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

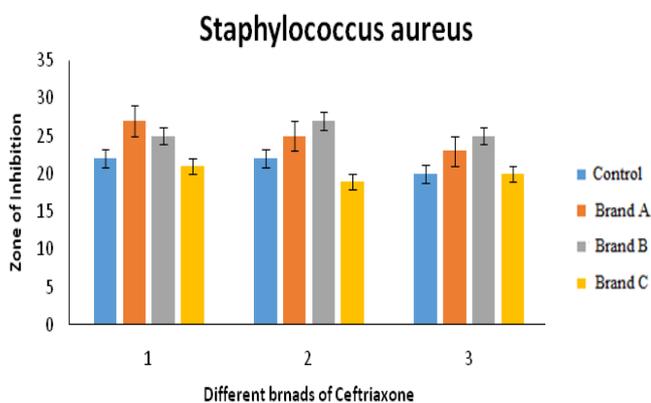


Figure 1 Effect of three ceftriaxone brands against *Staphylococcus aureus*.

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

For the bacteria *Klebsilla Pneumonia*, the mean diameter of inhibitory zone for control was 25±3.0mm. Furthermore, the diameter of inhibitory zone for (A) was 33mm. The mean diameter of inhibitory zone for Ceftriaxone (B) was 29±6.55mm. The mean diameter for the zone of inhibition Ceftriaxone (C) was 31.6±1.52 mm (Table 3, figure 3).

Table 2 Zone of inhibition of three brands of ceftriaxone against *Streptococcus pyrogens*

Dose of Ceftriaxone 30µg	<i>Streptococcus pyrogens</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

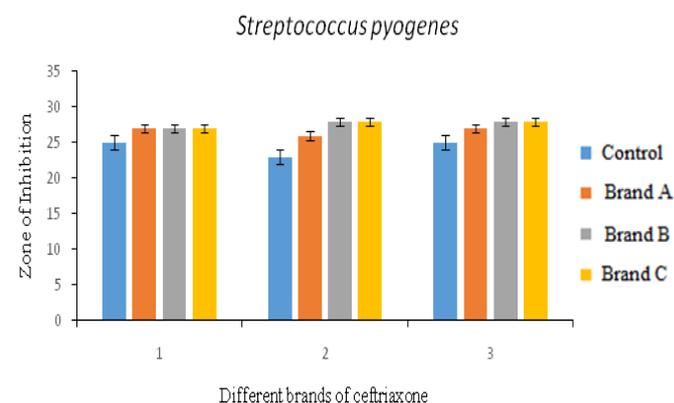


Figure 2 Effect of three ceftriaxone brands against *Streptococcus pyrogens*.

Table 3 Zone of inhibition of three brands of ceftriaxone against *Klebsilla pneumoniae*

Dose of Ceftriaxone 30µg	<i>Klebsilla pneumoniae</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

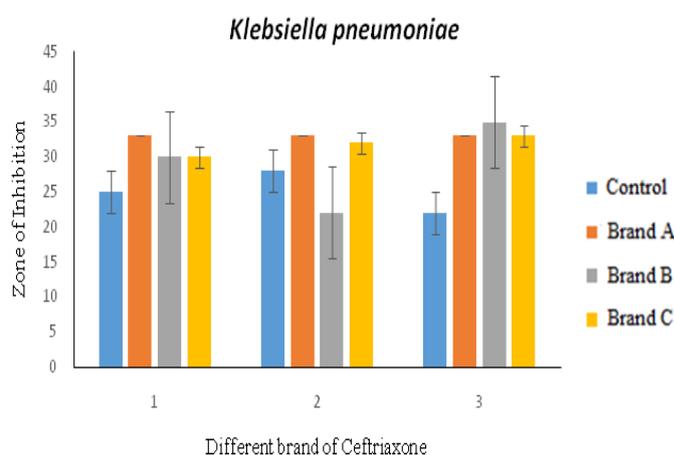


Figure 3 Effect of three ceftriaxone brands against *Klebsilla pneumoniae*.

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 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

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 (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.

Acknowledgments

None.

Conflicts of interest

Authors declare that there is no conflict of interest.

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