***Original Research Article***

# Ceftriaxone Injectables: An InVitro Evaluation of Antimicrobial Activity and Comparative Efficacy


## ABSTRACT

**Aims:** The objective of the current study was to compare the In Vitro efficacy of regionally produced brands of ceftriaxone with that of the global innovator brand. Therefore, a fact-based conclusion at the end of the study will serve to dispel any uncertainty regarding the efficacy of medications produced locally, whether they are inferior or equal in their comparative activity.
**Study design:** This study was In Vitro Lab based study.
**Place and Duration of Study:** The study was performed in Riphah International University, Department of Pharmacy from July 2021 to August 2021.
**Methodology:** Five regional brands and one international parent brand (Rocephin) were chosen. The multinational product was regarded as the standard. *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aurogenosa* were the four bacterial strains against which activities were tested. Antibiotics' zone of inhibition was measured using the agar well method, and their minimum inhibitory concentration was identified using the broth dilution method.
**Results:** All tested brands showed effectiveness against *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aurogenosa.*Some of the brands at some point showed more In Vitro efficacy than the standard brand.
**Conclusion:** Health care professionals can be confident in prescribing locally manufactured brands that have equivalent efficacy to their international counterparts based on the findings of the study. Healthcare professionals can suggest the generic brand in population where medicine cost is directly affecting the patients adherence to therapeutic regimen.



*Keywords: Ceftriaxone, Bacterial Strains, Minimum Inhibitory Concentration, Drug substitution, Microbial resistance, Healthcare Professionals, Agar Well Method, Broth Dilution Method.*

## 1. INTRODUCTION

Microbial drug resistance is one of the most important problems associated with overuse and abuse of antimicrobial agents in clinical settings [1]. As a result, antibiotics efficacy is compromised upon the emergence of the antibiotic-resistant microbes [2]. Many developing countries still lack an adequate mechanism for checking the quality of generic pharmaceuticals on the market, which contributes to the spread of substandard or counterfeit drug products [3]. As a result, validation of the analysis is essential to ensure the trustworthiness of the data obtained in the quality testing of pharmaceutical products [4].

Ceftriaxone is the third generation antibiotic that belongs to a class of cephalosporin antibiotic [5]. Ceftriaxone has a broad spectrum of activity, which includes Gram-positive and Gram-negative aerobic and some anaerobic bacteria [6]. This includes its use in meningitis, pneumonia, endocarditis, pelvic inflammatory disease, and infections of the middle ear, bone, joint, and urinary tract [7]. Ceftriaxone sodium is available as parenteral dosage form and administered Intra-venously or Intra-muscularly. WHO has listed Ceftriaxone as an essential medicine, and it is one of the widely used third-generation cephalosporin in Pakistan [8-10]. However, the prices of accessible brands vary too much, and considerable consideration should be given to policy formation in order to boost the affordability of medicines [11]. Due to Pakistan's rapidly expanding population and younger age group, the healthcare sector is under significant financial strain [12]. But there are legitimate concerns, and some data suggests that generic formulations may not save the patient or the health care system as much money as expected [13].

Generic versions of ceftriaxone have been readily available in several countries since Rocephin patent in Europe expired in 2000. Proof of bioequivalence between generic and innovator products ensures the safety and efficacy of generic brands [14]. To be considered a generic drug, the formulation must be identical to that of an existing drug and have the same amount of the active ingredient [15]. Many generics of ceftriaxone fail to match the branded product's quality criteria, according to past studies in Japan and England. Another trial found a decrease in efficacy and an increased probability of clinical failure [16-18]. Study in Ethiopia, on the other hand, found that the efficacy of generic brands of Ceftriaxone was equivalent to that seen in the parent version of the drug [19]. Physicians and patients alike are concerned about the quality and efficacy of antibiotics made by both global and local pharmaceutical companies.

According to our knowledge, no comparison of the efficacy of local brands of Ceftriaxone with an innovator brand has been published in Pakistan. The purpose of the study presented in this paper is to debunk medical professionals' concerns regarding the efficiency of generic antibiotics by comparing four different strains of bacteria to the innovator brand of routinely prescribed injectable ceftriaxone available in Pakistan. It was important for us to look at the Minimum Inhibitory Concentration (MIC) of various generics included in our research to give medical professionals with evidence-based knowledge they can use.

## 2. MATERIALS AND METHODS

### 2.1 Sample Preparation

Ceftriaxone injections were obtained from local pharmacies for this investigation. – In all, there were six generics utilized, five of which were made locally and one was made by a multinational, namely Rocephin, to which the efficacy of the local generics was compared. Cef-1 to Cef-6 were the generic versions of ceftriaxone, with Cef-1 being the worldwide brand and regarded the standard for the rest of the generics. Antibiotics were diluted to a final concentration of one percent (1%) in water for Injection for testing.

### 2.2 Microorganisms

Four bacterial strains, namely, *Staphylococcus aureus* (ATCC: 29737), *Escherichia coli* (ATCC: 6633), *Pseudomonas aeruginosa* (ATCC: 25619) and *Staphylococcus epidermidis* (ATCC: 12228) were included in this study. The microorganism's selection was based on the availability of strain in the laboratory and was considered as test microorganisms for comparative evaluation of ceftriaxone brands. All microorganisms were grown in broth and incubated at $35^{o}$C for 24 hours. These inoculums were used for the bioassay.

### 2.3 Anti-Bacterial Assay

The antibacterial activities of different injections of Ceftriaxone were investigated by agar well plate diffusion method [20]. Agar plates were prepared by pouring sterile nutrient agar media in plates and allowed to solidify. These were then incubated at $35^{o}$C for 24 hours to confirm the sterility by checking the absence of any bacterial growth. Then each bacterial strain included in the present study was swabbed on separate plates. Wells of respective diameter were then

made on plates using sterile sharp tubes, and diluted samples of ceftriaxone were added. Then agar plates were allowed to incubate at 35$^{o}$C for 24 hours. After the incubation period, the zone of inhibition (ZOI) was measured by using scale and later compared. ZOI was measured in millimeter (mm).

### 2.4 Minimum Inhibitory Concentration

There are several methods to observe the minimum inhibition concentration (MIC) of antibiotics [20]. Among them, macro-broth or tube dilution method is one of the common methods of antimicrobial susceptibility testing [21]. We prepared two-fold dilutions of antibiotics i.e. 0.5, 1, 2, 4, 8, 16, and 32 µg/mL in liquid growth medium. The test tubes containing antibiotic were then inoculated with 1-5 x $10^5$ CFU/ml bacterial suspensions. Tubes were then incubated at 35$^{o}$C for 24 hours. Tubes were examined for visible growth of bacteria as evidenced by turbidity. The lowest antibiotic's concentration that stopped the bacterial growth was considered as the MIC.

## 3. RESULTS

An antimicrobial susceptibility profile was aimed at in this investigation. The results of the zone of inhibition were recorded in millimeters (mm). The activity of all generics brands against different bacterial isolates is discussed below.

Antimicrobial activity against *Escherichia coli* (ATCC: 6633) is shown in figure 1 (a and b). The activity was performed in duplet, and their diameters were measured (Table 1). The results showed that average results of Cef-5 were higher i.e. 22mm as compared to other brands. Other activities recorded were 20mm for Cef-4, 19.5mm each against Cef-1 and 3, 18mm against Cef-6 and 12.5mm against Cef-2 (Fig.2).

Antimicrobial activity of generic Ceftriaxone against *Staphylococcus epidermidis* (ATCC: 12228) is shown in figure 1 (c and d). Activities in duplet are shown in table 1. The result showed that maximum ZOI recorded was 21mm for Cef-4. While ZOI of other generics was 20mm for Cef-2, 19.5mm for Cef-3, 18.5mm for Cef-5, 17.5mm for Cef-1 and 5mm for Cef-6 (Fig.2).

Antimicrobial activity against *Staphylococcus aureus* (ATCC: 29737) is shown in figure 1 (e and f). ZOI's are shown in table 1. The results showed that Cef-4, i.e., 18.5mm, showed maximum ZOI. ZOI of others was, 17mm by Cef-1, 15mm by Cef-5, 13.5mm by Cef-3, 7.5 mm by Cef-2 and 5.5mm by Cef-6. Figure 2 shows the average ZOI comparison in graphic form.

Antimicrobial of generics of Ceftriaxone against *Pseudomonas aeruginosa* (ATCC: 25619) is shown in figure 1 (g and h). The ZOI of duplet, along with average ZOI, is shown in table 1. The result showed that maximum ZOI was observed in Cef-5, i.e. 23mm. Other ZOI witnessed were 17.5 mm by Cef-1, 17 mm by Cef-4, 15.5 mm by Cef-3 and Cef-6 while 13.5 mm by Cef-2. Figure 2 shows the average result of the zone of inhibition.

The MIC was calculated for all the generics included in the present study (Table 2).

MIC against *Escherichia coli* showed that MIC of Cef-5 was 16µg/ml, Cef-1, Cef-2 and Cef-4 were 32µg/ml while MIC of Cef-3 and Cef-6 were >32 µg/ml.

MIC against *Pseudomonas aeruginosa* showed that MIC of Cef-5 and Cef-6 were 16 µg/ml. MIC of Cef-1, Cef-3, and Cef-4 was 32 µg/ml while Cef-2 showed MIC of >32 µg/ml.

MIC against *Staphylococcus epidermidis* showed that the MIC of Cef-4 was observed to be 16µg/ml. MIC of Cef-1, Cef-3, and Cef-5 was 32 µg/ml. MIC of Cef-2 and Cef-6 was found to be >32µg/ml.

MIC against *Staphylococcus aureus* showed that MIC of Cef-1, Cef-2, and Cef-3 was observed 32µg/ml. The MIC of Cef-4, Cef-5 and Cef-6 were found to be >32 µg/ml.

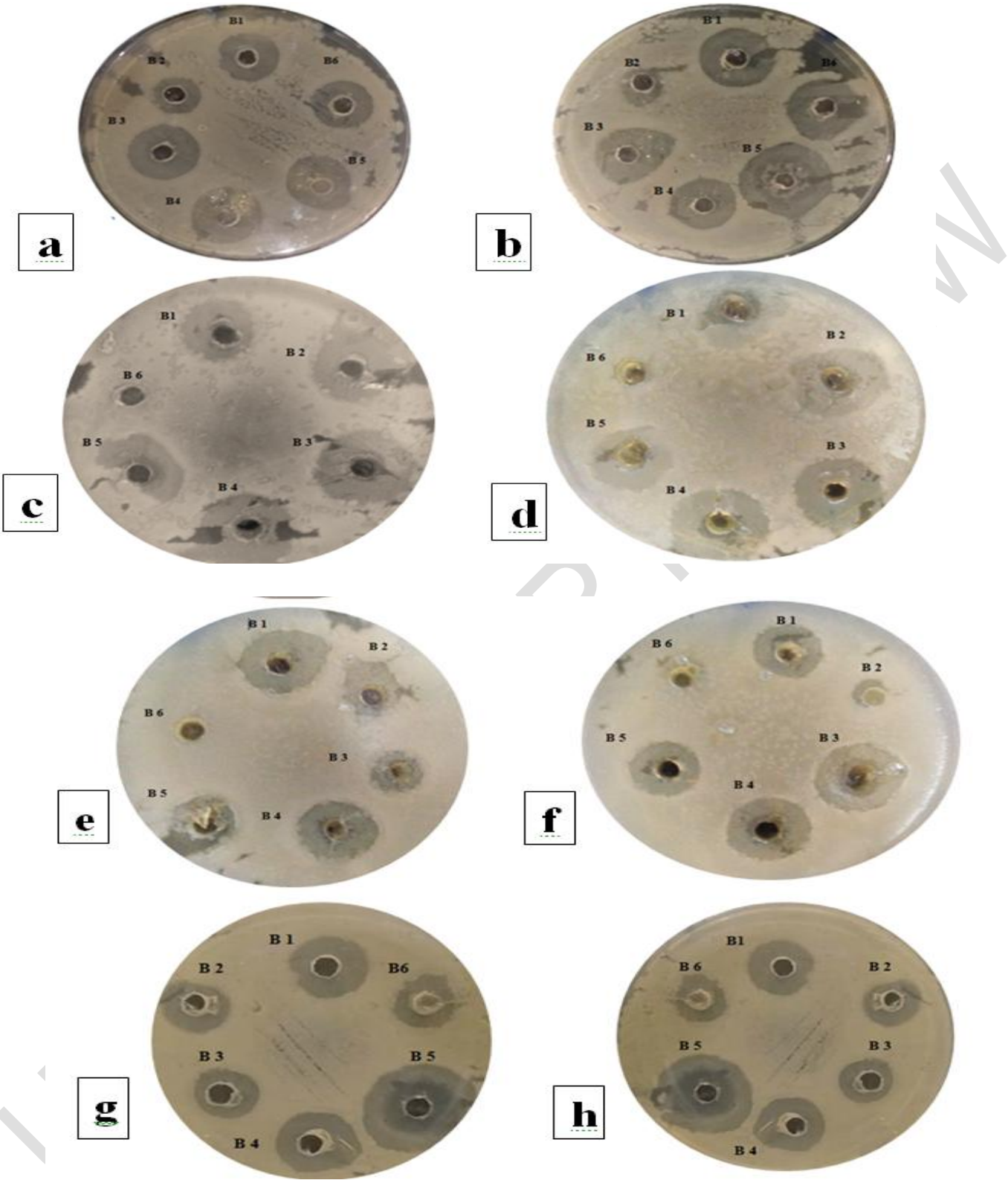


**Figure 1:** Ceftriaxone brands zone of inhibition: a and b shows activity against *Escherichia coli*; ATCC:6633 , c and d shows activity against *Staphylococcus epidermidis*; ATCC:12228, e and f shows activity against *Staphylococcus aureus*; ATCC:29737, g and h

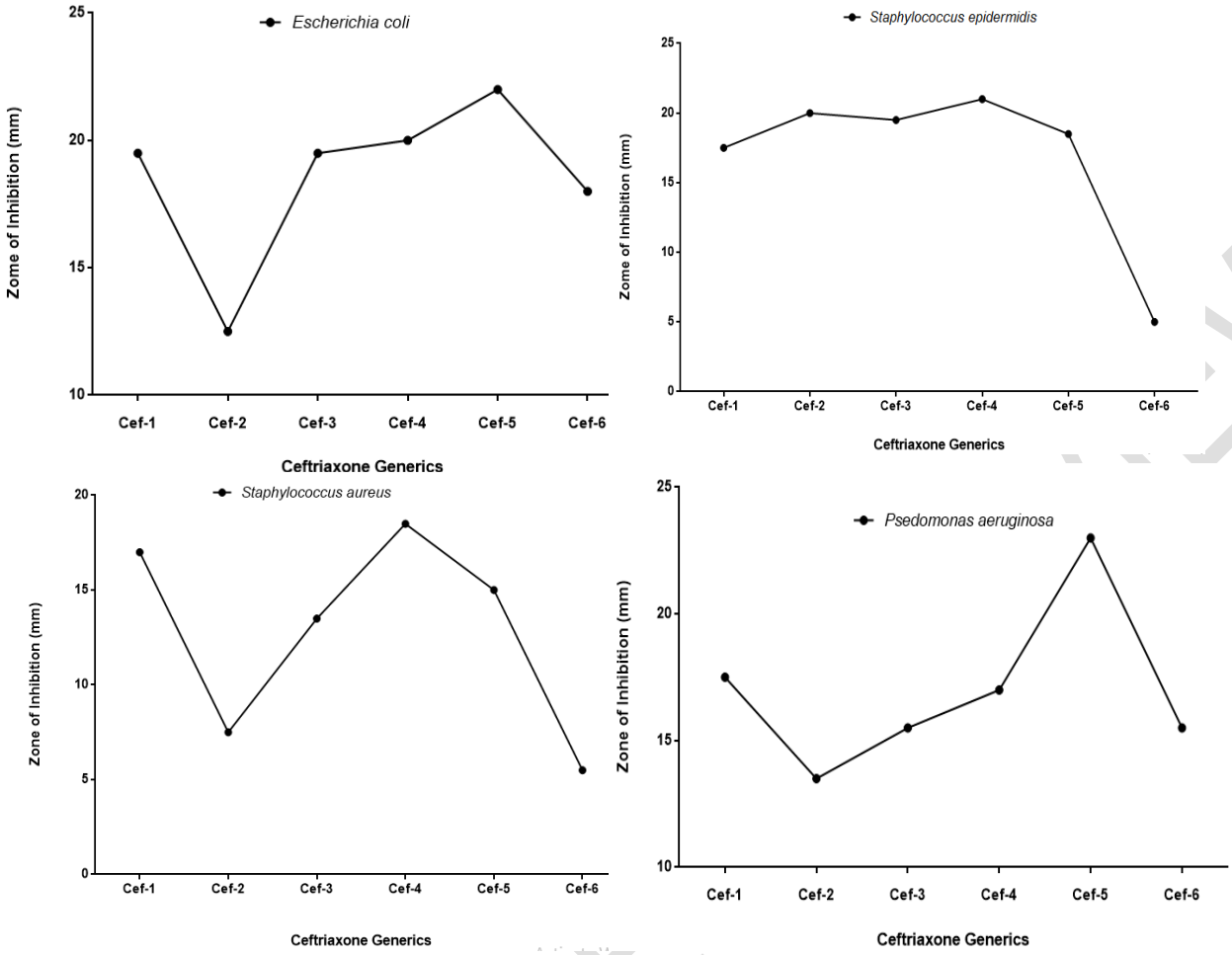


**Figure 2:** Ceftriaxone brands average zone of inhibition against the respective strains in graphic form

**Table 1:** Ceftriaxone brands zone of inhibition against *Escherichia coli* (E.coli) ; ATCC: 6633, *Staphylococcus epidermidis* (S. epidermis) ; ATCC: 12228, *Staphylococcus aureus* (S.aureus) ; ATCC: 29737 and *Pseudomonas aeruginosa* (P.aeruginosa) ; ATCC: 25619

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| Code No. | Brands | Results (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | | | *S. epidermidis* | | | *S. aureus* | | | *P. aeruginosa* | | |
| | | 1ST | 2ND | Avg. | 1ST | 2ND | Avg. | 1ST | 2ND | Avg. | 1ST | 2ND | Avg. |
| B 1 | Cef-1 | 20 | 19 | 19.5 | 20 | 15 | 17.5 | 19 | 15 | 17 | 18 | 17 | 17.5 |
| B 2 | Cef-2 | 13 | 12 | 12.5 | 20 | 20 | 20 | 9 | 6 | 7.5 | 13 | 14 | 13.5 |
| B 3 | Cef-3 | 19 | 20 | 19.5 | 19 | 20 | 19.5 | 10 | 17 | 13.5 | 15 | 16 | 15.5 |
| B 4 | Cef-4 | 20 | 20 | 20 | 21 | 21 | 21 | 17 | 20 | 18.5 | 17 | 17 | 17 |
| B 5 | Cef-5 | 23 | 21 | 22 | 15 | 22 | 18.5 | 15 | 15 | 15 | 24 | 22 | 23 |
| B 6 | Cef-6 | 20 | 16 | 18 | 5 | 5 | 5 | 6 | 5 | 5.5 | 15 | 16 | 15.5 |

**Table 2:** Ceftriaxone brands Minimum inhibitory concentration *Escherichia col*i ; ATCC: 6633, *Staphylococcus epidermidis* ; ATCC: 12228, *Staphylococcus aureus* ; ATCC: 29737 and *Pseudomonas aeruginosa* ; ATCC: 25619; µg/ml (microgram per millileter)

| Brands | *Escherichia coli* | *Pseudomonas aeurogenosa* | *Staphylococcus epidermidis* | *Staphylococcus aureus* |
|---|---|---|---|---|
| Cef-1 | 32 µg/ml | 32 µg/ml | 32 µg/ml | 32 µg/ml |
| Cef-2 | 32 µg/ml | >32 µg/ml | >32 µg/ml | 32 µg/ml |
| Cef-3 | >32 µg/ml | 32 µg/ml | 32 µg/ml | >32 µg/ml |
| Cef-4 | 32 µg/ml | 32 µg/ml | 16 µg/ml | 32 µg/ml |
| Cef-5 | 16 µg/ml | 16 µg/ml | 32 µg/ml | >32 µg/ml |
| Cef-6 | >32 µg/ml | 16 µg/ml | >32 µg/ml | >32 µg/ml |

## 4. DISCUSSION

Antibiotic resistance among bacteria is becoming an unavoidable fact, posing a serious threat to the efficacy of currently available therapeutic approaches [22-24]. The microbes become resistant due to the overuse and misuse of these antimicrobial agents. The success of the antimicrobial therapy relies heavily on microorganism's susceptibility towards available treatment. The present study was performed to observe the sensitivity of microbes and comparison of the effectiveness of different generics of Ceftriaxone.

The utilization of Ceftriaxone has increased for most infections resulting from Gram-positive and negative aerobes and anaerobes [25-27]. This study shows that Ceftriaxone is still useful against these microorganisms, and all of the generics showed effectiveness against them.

In the case of *Escherichia coli,* all of the tested brands showed efficacy against the bacterial strains. This is in agreement with the previous study, where researchers found that Ceftriaxone showed effective results against *Escherichia coli [28].* Similarly, in the case of *Staphylococcus epidermidis*, Cef-1 to Cef-6 all showed their respective efficacy. Previously researchers also found that Ceftriaxone is also effective against *Staphylococcus epidermidis* [29]. In the case of *Staphylococcus aureus,* efficacy was observed which is alligned to past study Ceftriaxone also showed effectiveness against *Staphylococcus aureus* [30]. In the case of *Pseudomonas aeruginosa,* In Vitro evaluation showed their effectiveness. A previous study by Knapp et.al. relates where it was found that Ceftriaxone showed effectiveness against *Pseudomonas aeruginosa [31].* Magnus et.al. also concluded in their study that In Vitro effectiveness of ceftriaxone was better than the other salt against the bacterial strains [32].

Ceftriaxone generics are just as effective as the brand-name version in treating patients in the local area. The findings of the executive insights of locally and multinational companies study also suggest that local brands are effective, but on the other hand, multinational brands has a good brand image [33]. Hence, we can use local brands against the above stated bacterial infection wherever it is possible. According to Luga et.al. therapy compliance and costs will be reduced as a result of this strategy in which generic prescribing is encouraged [34].

Similar studies could be performed in the future for comparing the efficacy of the brands of local manufactures for other drugs with the innovator products, which may help analyze the quality of local brands.

## 5. CONCLUSION

Locally manufactured brands are equally effective as the multinational Ceftriaxone brand. Some of the generics even showed more ZOI and MIC against bacterial strain as compared to standard. Based on the results of the present study, it can be safely said that health care professionals can prescribe the locally manufactured brand having equal and comparable efficacy to their multinational counterpart. Increased local medicine prescriptions will also aid in stabilising the nation's overall economy. Additionally, there is a price difference, allowing patients to receive effective treatment against microbial illnesses that respond to ceftriaxone at a reasonable cost. Adherence to therapeutic regimens will result in patients for whom cost of therapy matters.

## Ethical Approval

Ethical permission of the study was obtained from Riphah Institute of Pharmaceutical Sciences and study was performed in laboratory in Riphah Institute of Pharmaceutical Sciences.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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