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*(Article)*

# Does the price matter?
# potency comparative study for different Ceftriaxone generics in the Egyptian market

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**ABSTRACT**

There are a lot of ceftriaxone sodium generics in the Egyptian market with a huge variation in prices, this raises a tremendous question mark, does the difference in prices reflect a difference in potency? So, we made a small-scale experiment to test generics potencies using the antibiotic agar diffusion method similar to the antibiotic assay method with its graphical and mathematical calculations. This experiment was in a form of a comparative study of ten ceftriaxone sodium generics in the Egyptian market using antibiotic agar diffusion against a standard strain of

*Staphylococcus aureus* ATCC 977 as evaluator microorganism to test the antimicrobial effect of each generic by measuring Inhibition zone diameter (IZD) they leave on pre-inoculated agar plates; Considering the highest priced generic we purchased from the Egyptian market, which was generic A as a reference substitute for pure ceftriaxone sodium which couldn't be afforded in this study. Our results revealed that there was a significant difference in potency, but curiously those differences were not correlated to the prices of the generics.

*Keywords:* Ceftriaxone sodium; Antibiotic resistance; Egyptian market; antibiotic agar diffusion method; potency comparison.

## 1-Introduction

Antibiotics were first introduced in the middle of the past century and they caused a rise in their fame for treating high and severe bacterial infections. Thus, in the past fifty or sixty years, it has been one of the expectations of physicians that antibiotics would be the cure for all bacterial-infected patients. That's why patients believe that they are the miracle drugs that cure it all, which consequently leads in a way or another to antibiotic misuse (1). But, that doesn't contradict that antibiotic has a great impact as prophylaxis, not just a post-infection treatment as in surgical operation to prevent site infection (2).

Ceftriaxone sodium which is a β-lactam antibiotic from the third generation is a semi-synthetic cephalosporin that has a bactericidal action through inhibition of cell wall synthesis, it also has high stability against most β-lactamases. It is delivered from a fermentation product. It was synthesized through the precursor 7-amino-cephalosporanic acid (7-ACA). Ceftriaxone sodium is present in the disodium hemi pentahydrate form, **Figure 1**, it's used for the parenteral route because it's not absorbed by the oral route, its main significance is that it can cross the blood-brain barrier, while old generations can't.

Its activity is of a broad type against Gram-positive and Gram-negative bacteria even against first- and second-generation cephalosporin-resistant Gram-negative bacilli. The activity against Gram-positive is by no means smaller than the first-generation cephalosporins. Its range of activity is increased against Gram-negative bacteria, and it is also capable of acting against Gram-negative bacilli which are resistant to first- and second-generation cephalosporins. It is prescribed in cases of many diseases such as septicemia and meningitis (3).

Antibiotic misuse can lead to the increase of bacterial resistance, which happens when the wrong way of using the antibiotics leads the bacteria to acquire new genetic traits thus acquiring resistance towards antibiotics. The abuse of such dangerous products greatly affects the survival of the human race. Therefore, leading to the utmost need to find new replacements for antibiotics that are equally effective or even more effective, just as how antimicrobial peptides (AMPs) are being nominated as one of the best choices of antibacterial agents these days. Therefore, it is an urgent need to find effective alternatives to antibiotics. Antibiotic abuse is a serious case that is happening worldwide leading to hefty casualties due to its side effects, and became so fierce that it started to lay its fangs on innocent patients all across the globe (4), (5).

Our choice for ceftriaxone sodium to be the antibiotic we test in this experiment was not only because of the high generics number you can find from ceftriaxone in the Egyptian market, but also the fatal misuse of ceftriaxone in Egypt as to be used commonly for any viral combined with little grade fever such as influenza and even common cold which make some pharmacists sarcastically says that "Fever is not one of the symptoms for ceftriaxone deficiency". Finally, that's will not remain funny for a long time soon which will lead to ceftriaxone resistance in Egypt we didn't take the ceftriaxone misuse problem seriously.

Microorganisms resist antibiotics with different mechanisms that include active efflux of a drug, lowering drug entry into the cell, changes in gene expression, modifying drug targets, and mutational adaptation. Moreover, Resistance to antibiotics can occur due to more complex phenotypes like biofilm formation and quorum sensing.

Gonococci acquire ceftriaxone resistance through resisting plasmid that produces B-lactamase (this mechanism is quick and easily passed through strains) and gradual accumulation of mutation genes responsible for resistance in chromosomal genes, and this happens for a long time. In contrast, *E.coli*'s most important resistance mechanism against ceftriaxone is extended-spectrum B-lactamase. Also, *Salmonella*'s resistance to ceftriaxone is accompanied by producing CMY-2 (64%) and CTX-M-3 (27%) β-lactamases. *Neisseria gonorrhoeae* strain's resistance to ceftriaxone is due to chromosomal mutations in penA, mtrR, and penB (6).

All across the globe antimicrobial resistance has been on the rise, but in Egypt data are sparse, so no specific data was showing that all types of microbes have shown resistance to ceftriaxone but studies of antimicrobial susceptibility patterns of bloodstream isolates of Gram-positive cocci and Gram-negative bacilli in five hospitals in Cairo, Egypt, from 1999 to 2000. In

addition, susceptibilities of non-bloodstream isolates of *Streptococcus pneumoniae* and *Enterococcus* spp. were analyzed, where high rates of resistance were found in most of the bacteria studied, proving that microbes have acquired and highly developed resistance that aligns with the recent studies, which their own indicates the horrible future (7).

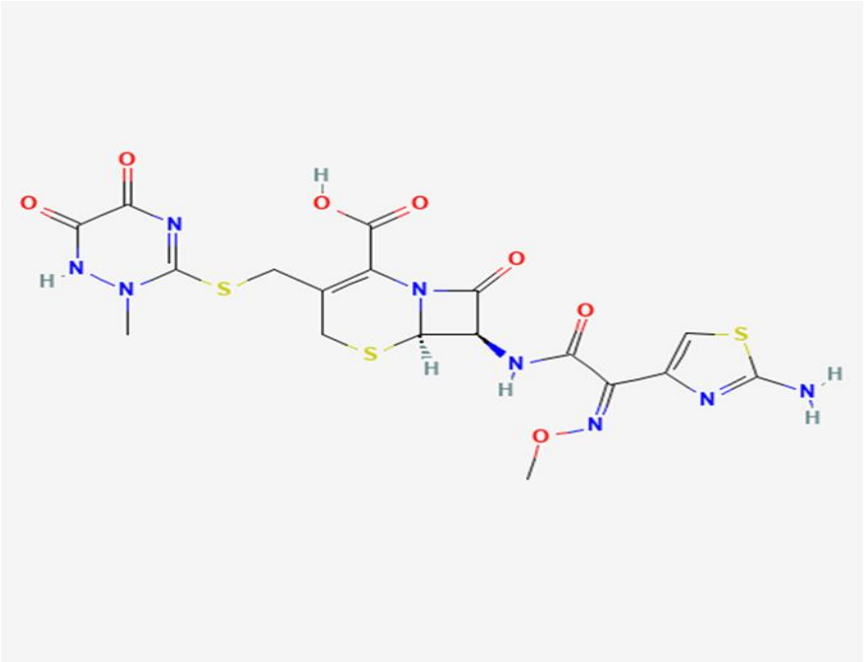

**Figure 1:** Ceftriaxone sodium chemical structure.

PubChem Identifier: CID 5479530

URL: https://pubchem.ncbi.nlm.nih.gov/compound/ceftriaxone#section=2D-Structure

## 2. Experimental

Materials used were ceftriaxone sodium vials for intramuscular injection from 10 different generics in the Egyptian market, alphabetically named A, B, C, D, E, F, G, H, K, and L considering A ceftriaxone generic as a standard for comparison with other generics due to its price as a substitute for pure ceftriaxone we couldn't handle to get, then we prepared three dilutions of each 0.125, 0.25 and 0.5 microgram per mL water testing their antimicrobial effect against 0.5 MacFarland dilution of *Staphylococcus aureus* ATCC 977. Tools were Petri dishes with 100mm outer diameter and 90mm inner diameter, Cork borer with a diameter of 1cm, 11ml Nutrient agar, 11ml agar-agar for each Petri dish, Pasteur pipette, and Sterile surgical blade.

The strain *Staphylococcus aureus* ATCC 977 was chosen as an experimental microorganism. As they are susceptible to ceftriaxone sodium and produce sharply defined

inhibition zones that allow accurate measurements. The strains were cultivated and preserved in Grove-Randall's 1 culture medium. The standard microorganism was prepared according to the Brazilian and USP Pharmacopeia method. Before use, the microorganism was developed in a slant medium (Grove-Randall's 1, at 35±2 °C for 24 hours.). The aseptically growing microorganism was suspended in a sterile solution of 0.9% NaCl a suspension of 25 ± 2% turbidity at 580 nm using an absorption cell (10 mm), with a sterile solution of 0.9% NaCl as blank. The Grove-Randall's 1 culture medium was inoculated with the standard suspension at 1% (v/v) to compose the upper layer in the plate at 48 °C (8).

The antibiotic agar diffusion method was applied with the 3×3 parallel line design (3 dilutions of the standard and 3 dilutions of the sample) according to the European and Brazilian Pharmacopeias after seeded inoculation of our microorganism in the agar, we made 6 cups with a 10 mm diameter, three for standard (generic A) dilutions and other three for test (other generics) dilutions with was 0.125, 0.25 and 0.5 microgram per mL respectively for standard and test each cup filled with 200 microliters of the dilution and left in the incubator for 24 hr. to measure the inhibition zone around each cup as in **Figure 2** (9, 10).

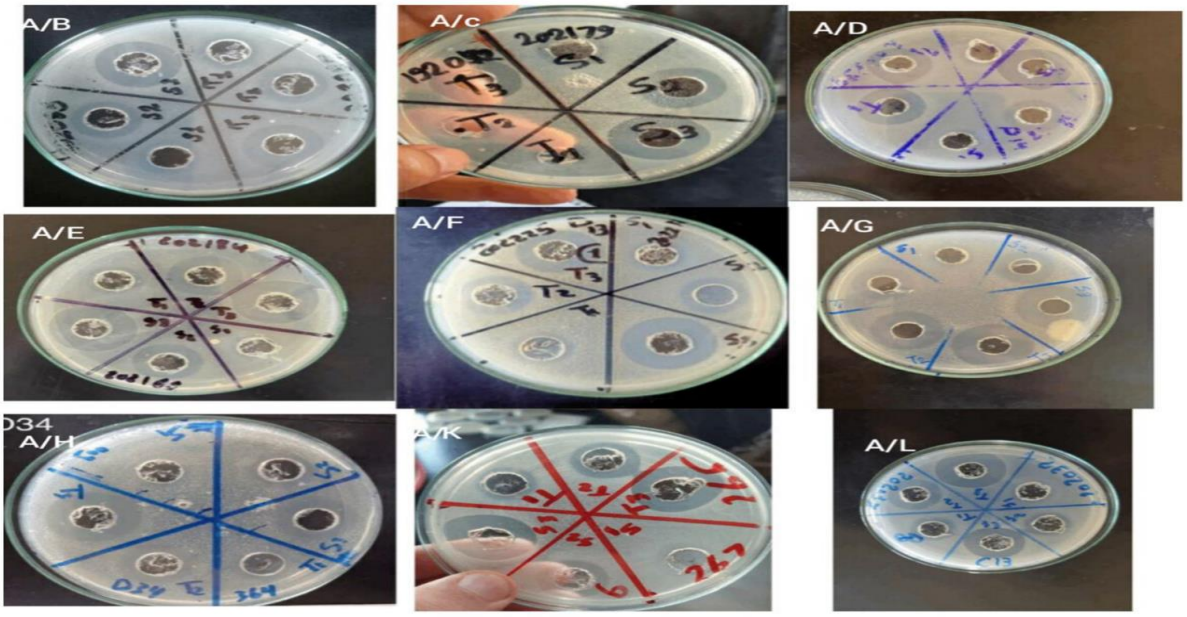


**Figure 2.** Antibiotic agar diffusion method against *Staphylococcus aureus* ATCC 977 as evaluating microorganism for ceftriaxone sodium antibiotic potency, each plate shows 6 inhibition zones 3 for generic A and 3 for the other generics B, C, D, E, F, G, H, K, and L respectively.

## 3-Results and Discussion

Five replicates have been done for each comparison of generic B, C, D, E, F, G, H, L, and K with our standard generic A; then, the inhibition zone diameter (IZD) of each antibiotic dilution (0.125, 0.25, 0.5) have been measured and the average was taken, represented as T1, T2, and T3 for generic B, C, D, E, F, G, H, K or L generics, while S1, S2 and S3 for generic A, **Table 1**.

A graphical illustration has been made for each antibiotic potency comparison using IZDs to reveal the difference in potencies and to visually detect variability between those generics, **Figure 3**. Also, we measured the potency percentage mathematically for more accuracy and we collectively compared between generics' potency percentage and prices, **Figure 4**.

By comparing the potencies, we can see a significant difference between ceftriaxone generics in the Egyptian market (Kruskal-Wallis chi-squared = 9, DF = 9, P-value = 0.4373) with generic B taking the first place by having the highest potency, followed by generics C, H, and E respectively, then came our standard generic A which had the highest price, finally, generics F, G, and L came with the lowest potencies respectively.

From a price perspective, you can see the wide price range from almost 55 L.E to around 25 L.E which is an unneglectable difference.

If we are considering only the two variables, potency and price in our consideration, the most cost-effective generic can be generic C which is giving a high potency relative to its price, then came generic H and generic B with a potency correlated to their prices.

**Table 1:** IZDs were taken and averaged for each ceftriaxone generic of the three dilutions (S1&T1: 0.125 μg/ml; S2&T2: 0.25 μg/ml; S3&T3: 0.5 μg/ml).

| **Ceftriaxone generic** | **S1 (mm)** | **S2 (mm)** | **S3 (mm)** |
|---|---|---|---|
| **A** | 16.60 | 21.00 | 26.28 |
| **Ceftriaxone generic** | **T1 (mm)** | **T2 (mm)** | **T3 (mm)** |
| **B** | 16.30 | 21.00 | 25.80 |
| **C** | 16.10 | 20.60 | 24.30 |
| **D** | 16.50 | 20.40 | 24.50 |
| **E** | 13.16 | 20.83 | 25.25 |
| **F** | 14.00 | 19.40 | 21.70 |
| **G** | 13.60 | 19.70 | 25.00 |
| **H** | 14.68 | 19.30 | 24.00 |
| **K** | 14.30 | 20.00 | 24.13 |
| **L** | 17.20 | 20.50 | 23.80 |

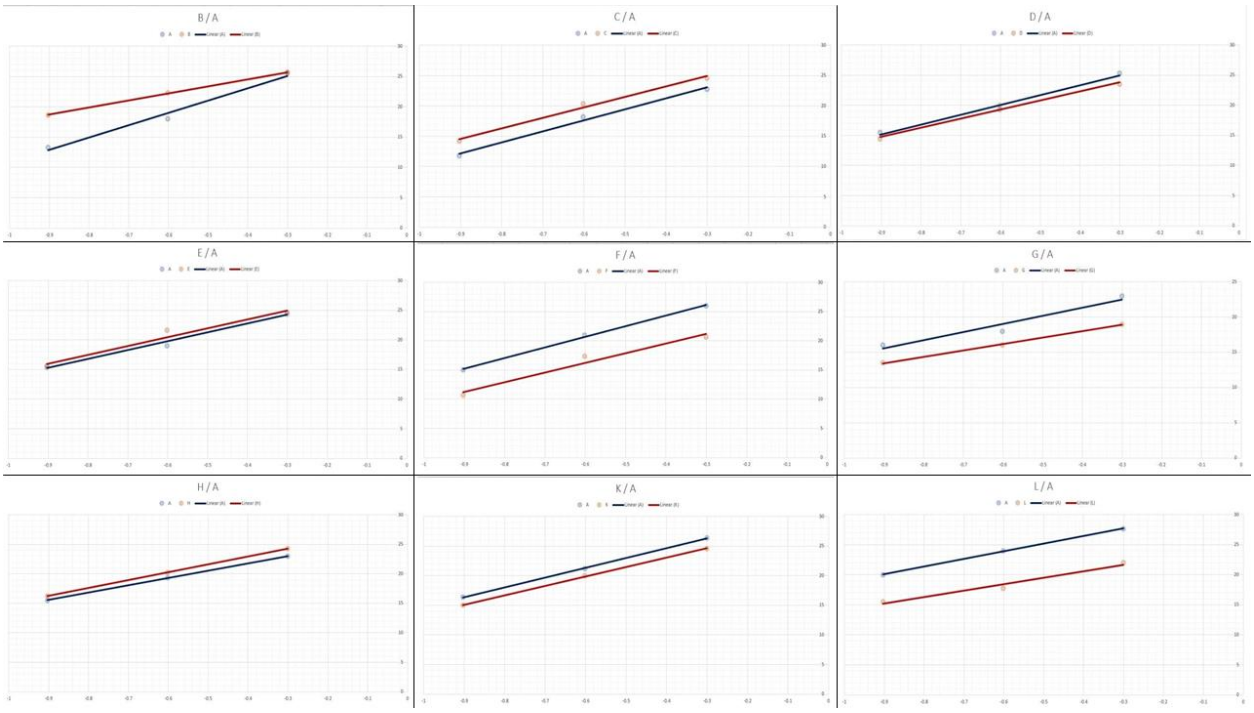


**Figure 3.** Those graphs show potency differences between drug A considered as reference drug generic A and the other 9 generics B, C, D, E, F, G, H, L, and K respectively.

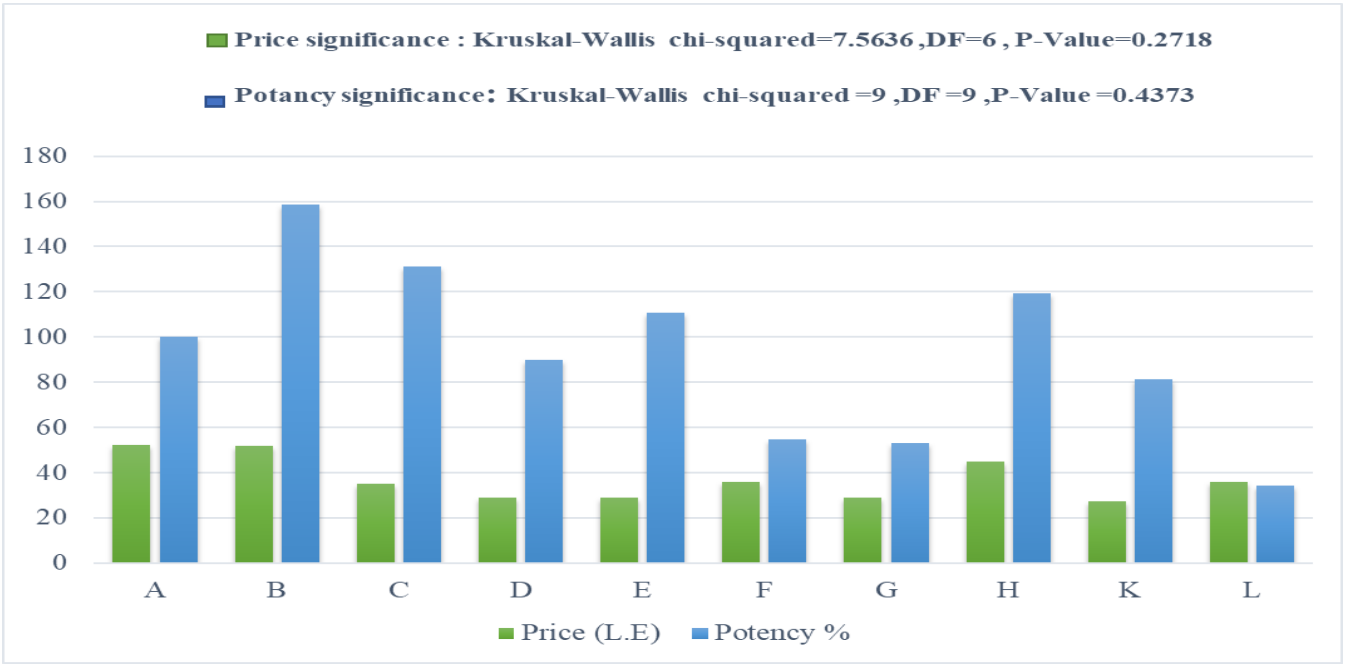


**Figure 4:** This chart shows the variation between the 10 generics of ceftriaxone according to their potencies and prices.

## 4. Conclusion

Based on the above results, if we excluded factors other than ceftriaxone active constituent potency like the impact of marketing, branding, production, transportation, packaging and other factors that affect the price of the drug, the wide variability in the prices which is not in all correlated to ceftriaxone potency can drive customers minds in Egyptian market due to the mistaken thoughts of the better quality you can get from the higher price; nevertheless, this was a small scale experiment and it needs to be done in a much larger scale with much more control in the factors like storage, transporting condition and other factors that can make an effect on ceftriaxone active constituent for more accurate result can be revealed to the public in the Egyptian market.

- **Conflict of Interest**

The Authors declare no conflict of interest.

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