Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(3), 5608-5615 | ISSN:2251-6727

Comparative In Vitro Activity of Ceftriaxone (Ceftriaxone) and Clarithromycin Formulations Against Standard Bacterial Strains

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(Received: 30 April 2026 Revised: 20 May 2026 Accepted: 10 June 2026)

KEYWORDS

wound healing; oral surgery; tooth extraction; suture technique; post-operative complications

ABSTRACT:

Background: The prevalent and irrational use of antibiotics, mainly Ceftriaxone (ceftriaxone) injection, has become public health concern that may be contributed to antibiotic resistance. In Libya, Ceftriaxone injection is commonly dispensed and prescribed in public pharmacies.

Aim: This study aimed to compare the antibacterial effect of different Ceftriaxone injection brands and Clarithromycin tablets available in the Libyan market against selected bacterial strains. Additionally, the study explored dispensing information related to these antibiotics in pharmacies across Tripoli, Libya, using a questionnaire survey.

Methods: brands of ceftriaxone injection and clarithromycin tablets were procured from licensed pharmacies. Antibacterial activity was assessed against such as, Staphylococcus aureus, ATCC 25923 25923, E. coli ATCC 25922, Salmonella ATCC 14028 and Shigella flexneri ATCC 12022 using the disk diffusion method. Zone diameters were measured in triplicate. A cross-sectional questionnaire assessed pharmacist prescribing patterns and dispensing data (101 sample size).

Results: Ceftriaxone formulations demonstrated significantly larger inhibition zones against all bacterial strains compared to clarithromycin ($p<0.05$). Ceftriaxone is dispensed frequently by 39.8% of participants, whereas, Clarithromycin is only 16.3% dispense it frequently. A majority of pharmacists (68%) reported that patients experience faster symptom relief with Ceftriaxone compared to Clarithromycin.

Conclusion: Marketed ceftriaxone formulations exhibited superior in vitro activity against the tested strains. Variability among brands underscores the need for post-market quality surveillance. This study displays the dominant use of ceftriaxone (Ceftriaxone) over clarithromycin among as samples of Libyan pharmacists are stated by participation in the questioner, this data supported by its supposed efficacy in treating respiratory infections and faster patient recovery

Introduction

Clinical microbiology and infectious disease treatment have been profoundly impacted by the worldwide epidemic of antibiotic resistance AMR, (Eccles, 2023; Nazir et al., 2025) Therapies lose predictable efficacy; the reliability of existing antibiotic agents has become a critical public health priority. Whereas, the mechanisms of resistance continue to under examined determinant of therapeutic success lies in the pharmaceutical quality. Commercial antibiotics, marketed under innovator brands, must meet stringent standards for identity, potency, purity, as well as dissolution. On the other hand, variations in manufacturing processes, excipient composition, packaging stability, and regional regulatory

Journal of Chemical Health Risks

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JCHR (2026) 16(3), 5608-5615 | ISSN:2251-6727

introduce measurable differences in active pharmaceutical ingredient ((Li et al., 2024; Tegegne et al., 2024). Antibiotics should only be prescribed for more serious bacterial infections or bacterial infections in people with immune system problems, as many infections get better on their own (Hansson & Irwin, 2022). Correct use of antibiotics is absolutely essential to help reduce antibiotic resistance. Bacteria become resistant to antibiotics over time, which makes them less effective. Furthermore, antibiotics can be taken by mouth as liquid, tablets, and capsules; also given via cream or ointment and injection (Bassetti et al., 2022).

There are various antibiotics available and each comes with different brand names, depending on the manufacturer (Hansson and Irwin, 2022). Antibiotics often administered belong to various pharmacological groups. Penicillin agents include phenoxymethylpenicillin, flucloxacillin and amoxicillin, while structurally similar cephalosporin include cefaclor, cefadroxil and cephalexin. Other important classes are tetracycline (tetracycline, doxycycline, lymecycline), aminoglycosides (gentamicin, tobramycin) and macrolides (erythromycin, azithromycin, clarithromycin) (Vilvanathan, 2021). Other clinically different choices are clindamycin, sulfonamide-trimethoprim regimens such as co-trimoxazole and mitroimidazole derivatives (metronidazole, tinidazole) (Adefegha, 2019). Fluoroquinolones (ciprofloxacin, levofloxacin, norfloxacin) target bacterial DNA replication whereas nitrofurantoin is largely used for coverage of uropathogens (Matei & Visan, 2025).

Ceftriaxone, a third-generation cephalosporin, is still an essential of moderated to severe bacterial infections. Bactericidal activity stems from high-affinity binding to penicillin-binding proteins, which disrupts peptidoglycan and which linked to compromises cell wall integrity. Despite its well characterized pharmacology, ceftriaxone is marketed globally under numerous brand names and generic designations. Parenteral formulations require strict control of pH, osmolality, and particle size to maintain solubility as well as sterility (Akhmetova & Zhaxylykova, 2025). Crystalline structure, moisture content, or stabilizing agents of subtle deviations can influence reconstitution kinetics which linked to subsequent serum concentrations. Furthermore, lactam efficacy is highly time-dependent (T>MIC), even minor reductions in bioavailable active pharmaceutical ingredient may compromise target attainment, in pathogens with borderline susceptibility (Laut et al., 2025).

Ceftriaxone has a long half-life and many other antibiotics may require multiple doses per day, which can be more cumbersome for patients and healthcare providers. Compared to some other antibiotics in its group, ceftriaxone has a relatively low risk of nephrotoxicity (kidney toxicity), which makes it safer for use in patients with compromised kidney function (Sharma et al., 2024). One another antibiotic is Clarithromycin which is macrolide antibacterial agent active in vitro and effective *in vivo* against the major pathogens responsible for respiratory tract infections in immunocompetent patients (Sharma et al., 2024). It is highly active *in vitro* against pathogens causing atypical pneumonia (*Chlamydia pneumonia*e, *Mycoplasma pneumoniae and Legionella spp*.) and has similar activity to other *macrolides against Staphylococcus aureus*, *Streptococcus pyogenes*, *Moraxella catarrhalis and Streptococcus pneumoniae*. *Haemophilus influenzae* is susceptible or intermediately susceptible to clarithromycin alone, but activity is enhanced when the parent drug and metabolite are combined *in vitro* (Garin et al., 2022). Therefore, regarding the finding of current study investigation evaluates the comparative in vitro antibacterial activity of commercially available ceftriaxone and clarithromycin formulations within reference bacterial strains. The selection of Clarithromycin for comparison was due to it is broadly used in Libya and can act as an alternative option in managing of some bacterial infections, assisting in reduction of unnecessary Ceftriaxone use and maintenance rational antibiotic use.

The research outcomes providing evidence-based insights for clinicians, pharmacists, regulatory authorities, and hospital procurement committees. In an era where antibiotic reliability is as consequential as resistance surveillance, ensuring that prescribed agents deliver consistent, guideline-concordant activity remains a foundational requirement for preserving therapeutic efficacy and mitigating the global AMR crisis.

Journal of Chemical Health Risks
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JCHR (2026) 16(3), 5608-5615 | ISSN:2251-6727

2. Material Methods

Commercially available antibiotic products were procured from licensed community pharmacies in Tripoli, Libya, during 2025. Two antibiotic classes were evaluated.

Ceftriaxone sodium for injection (1000 mg)

• Ceftriaxone sample: Ceftriaxone A 1000mg/ Ceftriaxone B1000mg/10ml.

Clarithromycin oral tablets

• Clarithromycin: Clarithromycin A 500mg/ Clarithromycin B 250mg

Clarithromycin oral tablets

• Clarithromycin: Klacid 500mg/ Claridar 250mg

Stock solutions were prepared under aseptic conditions. Ceftriaxone powder was reconstituted with sterile water for injection, then diluted in sterile dimethyl sulfoxide (DMSO; ≥99.9%, Sigma-Aldrich) to 10 mg/mL (Andrews, 2001). Clarithromycin tablets were pulverized, and an amount equivalent to 500 mg of active ingredient was dissolved in DMSO to achieve the same concentration. Then 30 µg/disc for ceftriaxone and 15 µg/disc for clarithromycin were freshly prepared by serial dilution immediately before testing (Abbey and Deak, 2019). Sterile cellulose discs, 6 mm diameter (Oxoid Ltd., UK), were impregnated with 10 µL of working solution and air-dried for 30 min.

Culture Media, Reagents, and Quality Assurance

All microbiological media were prepared following manufacturer protocols and sterilized by autoclaving (121°C, 15 min, 15 psi). Mueller-Hinton Agar (MHA; Oxoid Ltd., Basingstoke, UK) for susceptibility assays. Nutrient Broth (NB; HiMedia Laboratories, Mumbai, India) for inoculum propagation, 0.5 McFarland turbidity standard (bioMérieux, France) for inoculum standardization.

Dimethyl sulfoxide (DMSO; Sigma-Aldrich) as solvent and negative control. Cultures of bacteria were prepared through sterilizing nutrient broth, then inoculation was done with each bacterial strain at 37 °C for 18–24 hours. The antibacterial activity assessed by the disk diffusion method, after which the bacterial suspensions were spread over the agar surface by sterile cotton swabs. The sterile filter paper disks (6 mm in diameter) were soaked with different antibiotic samples dissolved in dimethyl sulfoxide (DMSO) using micropipettes and placed onto the inoculated plates, DMSO-soaked disks served as negative controls (H S et al., 2016). The plates were incubated at 37 °C for 24 hours, and the zones of inhibition surrounding the disks were measured in millimeters using a ruler.

Bacterial Strains

Table 1. Four reference strains of bacteria strain, obtained from the American Type Culture Collection (ATCC), served as test organisms

Source of Bacteria	ATCC Identifier	Gram Reaction	Representatives Infection Type
Staphylococcus aureus	25923	Positive	Skin/ soft tissue, bacteremia
Escherichia coli	25922	Negative	Urinary tract, sepsis
Salmonella	14028	Negative	Gastroenteritis, invasive disease
Shigella flexneri	12022	Negative	Bacillary dysentery

Journal of Chemical Health Risks
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JCHR (2026) 16(3), 5608-5615 | ISSN:2251-6727

Disk Diffusion Method

Overnight cultures in nutrient broth were adjusted to 0.5 McFarland turbidity (~1.5 × 10^8 CFU/mL), confirmed spectrophotometrically at 625 nm (Guzm Ã et al., 2018). Using a sterile cotton swab, the bacterial suspension evenly onto the surface of the MHA plates 90mm diameter. Antibiotic-impregnated discs (test formulations and reference standards) were aseptically positioned on inoculated agar using flame-sterilized forceps, maintaining ≥24 mm center-to-center spacing to prevent zone overlap. Then the plates were incubated aerobically at 35 ± 2°C for 16–18 h. Inhibition zone diameters (including disc) were measured to the nearest millimeter using calibrated digital calipers. Prepare sterile filter paper disks (6 mm diameter), add different samples of antibiotics in DMSO to the filter paper disks. Moreover, use the DMSO-soaked disks as negative controls and incubate the plates at 37°C for 24 hours. Measure the zones of inhibition (clear areas around the disks) in millimeters using a ruler. All brand strain combinations were tested in triplicate across three separate experimental days to account for inter assay variability (H S et al., 2016). The question form was designed to assess pharmacist prescribing patterns and brand awareness, this question form contains number of questions, which were answered by 101 participants, the social media and direct contacting was used to collect the answers during(15/April) to (16/May).

Statistical Analysis:

Calculate the average zone of inhibition for each sample of antibiotic and for each bacterial strain. Use a two-way ANOVA to compare the antibacterial activity of different samples for each bacterial strain using Graph pad prism8 software. All sets of data were based on a minimum of three separate experiments and p-values <0.05 were considered statistically significant.

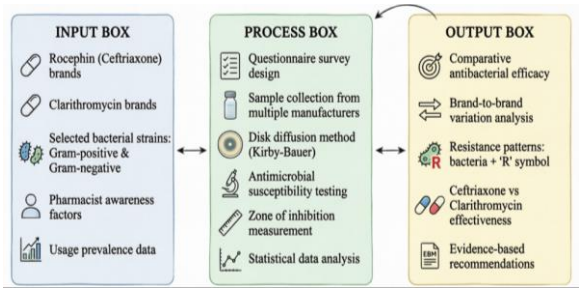

Figure (1) workflow diagram of the research study

Results

As mentioned in material and methods section, question form was designed to assess the prevalence of using Ceftriaxone and Clarithromycin in addition the awareness of pharmacists that participated, the results are presented in following

Frequency of Dispensing Ceftriaxone and Clarithromycin

Ceftriaxone is dispensed frequently by 39.8% of participants, while 36.7% dispense it occasionally. Rare dispensing was noted by 20.4%, and very few (3.1%) never dispense it. Clarithromycin is most often dispensed occasionally (49%), while only 16.3% dispense it frequently. The larger proportions of rarely (28.6%) and never dispensing (6.1%) compared to Ceftriaxone suggest that clarithromycin is used less regularly, possibly due to specific indications or prescriber preferences as presented in Figurer 2.

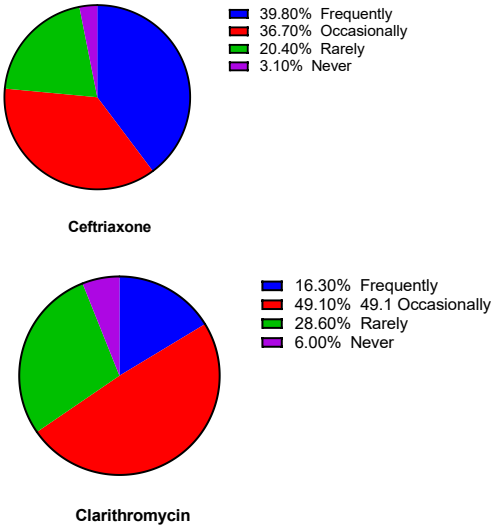

Figure 2 Frequency of Dispensing Ceftriaxone and Clarithromycin

Patient-Reported Symptom Relief

A majority of pharmacists (68%) reported that patients experience faster symptom relief with Ceftriaxone compared to Clarithromycin. This suggests that Ceftriaxone may provide quicker clinical improvement in many cases, possibly due to its broad-spectrum activity or injectable form. However, 15.5% noted Clarithromycin offers faster relief, and 16.5% stated

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there is no clear difference between the two as presented Figure 3. These perceptions may reflect varying patient responses, infection severity, or timing of treatment initiation.

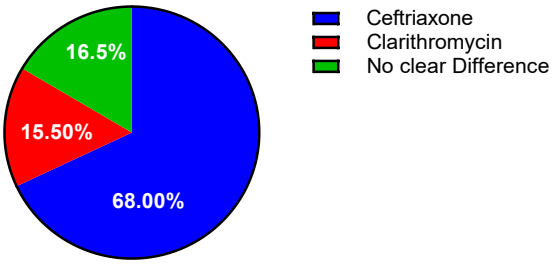

Figure 3 Patient-Reported Speed of Symptom Relief

The results of antibacterial effects have been demonstrating differences between in antibacterial activity and ceftriaxone and clarithromycin formulations, as well as brand to brand variability within each antibiotic class.

The antibiotic ceftriaxone A brand outperformed both brands of Clarithromycin against *E. coli* (gram negative), though all zones are relatively modest, which is possible intermediate susceptibility as presented in Figure 4.

On the other hand, ceftriaxone brand A showed significantly larger (double) inhibition zones 33mm than clarithromycin as shows Figure 4, which established ceftriaxone as the more suitable choice for *Salmonella* infections. Furthermore, the all antibiotic groups showed activity, but ceftriaxone brands produced significantly larger inhibition zones 34.7mm for *Staphylococcus aureus*. Whereas, clarithromycin B presented better than clarithromycin A, suggesting brand-specific formulation differences.

Pattern mirrors *Salmonella* results ceftriaxone brands are highly effective, while clarithromycin shows limited utility against *Shigella*

This study conducted that the antibacterial activity of ceftriaxone and Clarithromycin samples showed in Figure 4, confirmed differences in the inhibitory properties of both antibiotics samples ceftriaxone and clarithromycin on the tested bacterial strains. Both antibiotics displayed measurable antibacterial activity against the selected bacterial strains; however, the level of growth inhibition differ considerably depending on the type of microorganism used. The Ceftriaxone brands (A and B) show inhibition zone in range 20 to 34 mm on all bacterial strains used, whereas, brands of Clarithromycin (A and B) show inhibition zone 15 to 24mm.

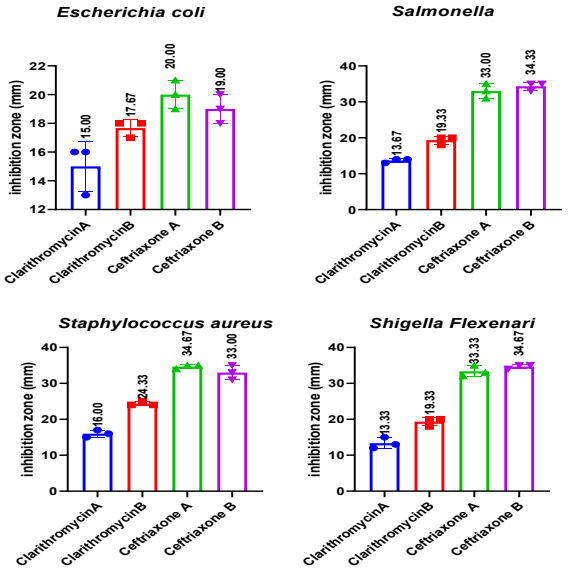

Figure 4 The antibacterial activity of Ceftriaxone and Clarithromycin samples on different bacterial strains

Cross-analysis of laboratory and survey data revealed a direct correspondence between in vitro potency and clinical utilization patterns. Ceftriaxone produced consistently larger inhibition zones against standard

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(3), 5608-5615 | ISSN:2251-6727

Gram-negative reference strains, which aligned with its higher prescribing frequency (39.8% frequent use) and pharmacist-reported faster symptom resolution (68%). Conversely, clarithromycin demonstrated a more restricted in vitro spectrum and was predominantly prescribed intermittently (49.0%), reflecting its narrower clinical indications and prevailing concerns regarding macrolide resistance. These concurrent findings indicate that prescriber preferences and perceived therapeutic outcomes closely track established susceptibility profiles, while remaining modulated by agent-specific pharmacodynamics and local resistance trends.

Discussion

The results of this study revealed that both antibiotics ceftriaxone and clarithromycin showed antibacterial effect against the selected bacterial strains; however, an important variation in activity was observed in all different pharmaceutical brands. This difference, as verified by variances in the zones of inhibition, shows that not all commercially available formulations have the same extent of antimicrobial effectiveness, although containing the same active pharmaceutical ingredient. Similar inconsistency in antibiotic brands has been described in previous studies, directing the concerns about therapeutic equivalence (Bate et al., 2009; Kelesidis & Falagas, 2015). One potential reason for this variability is the difference in the quality of formulation between brands. Factors such as the excipient composition, the manufacturing processes, purity of the active ingredient, and condition of storage could impact on the bioavailability and stability of antibiotics (Aulton & Taylor, 2013). Expired or corrupted formulations may cause reduction in antimicrobial effect as result of inadequate drug concentration or altered physicochemical properties (Newton et al., 2010). In the case of ceftriaxone, its act by inhibition of bacterial cell wall synthesis with interference with peptidoglycan cross-linking. Differences in effectiveness between antibiotics brands may reflect alterations in the stability of the β-lactam ring, which is crucial for its antibacterial action (Brunton et al., 2018). Similarly, clarithromycin, acting through inhibiting synthesis of bacterial protein by binding to the 50S ribosomal subunit. Any difference in drug dissolution or chemical integrity could influence its observed antimicrobial performance (Katzung & Vanderah, 2021). The detected differences among brands are agreed with results from previous studies, which documented changeability in antimicrobial effect between generic and branded antibiotics. This increases alarms about the clinical consistency of some formulations, mainly in regions where the regulatory oversight may be limited (Bate et al., 2009). Insufficient antibiotic strength can lead to treatment failure and encourage the development of antimicrobial resistance, a major global health concern (World Health Organization [WHO], 2020) (*Antimicrobial Resistance*, n.d.). It is also important to display some limitations of this study. The estimation was done through in-vitro methods, which might not completely reflect in vivo situations like drug absorption, distribution, metabolism, and immune response. Furthermore, the study tested limited number of bacterial strains and the absence of physicochemical analysis of the drug formulations. These outcomes emphasize on the importance of quality control and post-market surveillance of pharmaceutical products, particularly in regions with multiple available brands, to confirm the consistent in therapeutic efficacy. Consequently, ceftriaxone appears to be a more potent on the tested bacteria; however, clinical application should also consider factors such as resistance patterns, patient condition, and antibiotic stewardship guidelines. This aligns with previous findings that ceftriaxone is highly effective against Gram-negative and some Gram-positive pathogens. Clarithromycin, while effective against specific organisms (e.g., respiratory pathogens), showed lower inhibition in this study(Alshintari et al., 2026), possibly due to bacterial resistance or its narrower spectrum. Another aim of this study was evaluation of the prescribing patterns and dispensing data for two antibiotics samples among number of pharmacies through short questioner. The findings demonstrated in Figures 2 and 3 further validate the prevalent prescribing and dispensing practices of Ceftriaxone injections within public pharmacies in Tripoli-Libya. The outcomes show that Ceftriaxone is more frequently dispensed in community pharmacies compared with Clarithromycin. Approximately two-fifths of pharmacists who participate in this questioner stated frequent dispensing of Ceftriaxone however only a small proportion never dispense it, this reflecting its common clinical use and high prescribing rate. The broad-spectrum antibacterial properties of ceftriaxone, might be to the main reason in

Journal of Chemical Health Risks

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JCHR (2026) 16(3), 5608-5615 | ISSN:2251-6727

addition to it is effectiveness in treating a widespread range of bacterial infections. Several studies are in agreement with the findings of this study as about the higher dispensing frequency of antibiotics, mainly broad-spectrum antibiotic such as ceftriaxone, in community pharmacy settings. In United Arab Emirates study reported significant overprescribing and dispensing of antibiotics in community pharmacies, underscoring the prevalent use of broad-spectrum antibiotics in routine practice (Rabbani et al., 2023).

On the other hand, clarithromycin was largely dispensed occasionally, with higher extents of pharmacists answers with rare or no dispensing. The minor dispensing frequency of clarithromycin might be due to its specificity in therapeutic indications, and variances in physician prescribing preferences, and worries regarding antimicrobial resistance, or the accessibility of alternative oral antibiotics(Bassetti et al., 2022). Generally, the finding proposes a more confidence on Ceftriaxone in routine pharmaceutical practice, emphasizing on the need for constant monitoring of antibiotic dispensing patterns to maintenance balanced antibiotic use and diminish the risk of antimicrobial resistance(Laut et al., 2025).

Conclusion

The comparative evaluation of ceftriaxone and clarithromycin formulations showed that in general the Ceftriaxone demonstrates significant *in vitro* antibacterial activity against Gram-negative Enterobacteriaceae and selected Gram-positive cocci, with inhibition zone diameters aligning closely with Clinical and Laboratory Standards Institute interpretive criteria under controlled laboratory conditions. On other hand Clarithromycin, exhibits superior potency against streptococcal species and respiratory pathogens. The choice of suitable antibiotic is depended on several factors such as infection type and resistance patterns. Additional studies on real-world resistance trends and pharmacokinetics are recommended.

Funding: “This research received no external funding.”

Data Availability Statement: “The data are available at request.”

Conflicts of Interest: “The authors declare no conflict of interest.

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