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Research article

Evaluation Prepared Ceftriaxone Solution for Antimicrobial Susceptibility Testing of *Staphylococcus aureus* using the Agar Well Diffusion Method

Russul Taleb Zahy[1], Falah Hasan Obayes AL-Khikani*[1,2,3] Ali Abedulameer Alhusayni[2], Aya Aqil Abdul Hussein[1], Tabarak Ali Abdul Hussein[1].

1. Department of Medical Laboratory Technology, College of Medical Technology, The Islamic University, Najaf, Iraq
2. Department of Microbiology, Al-shomali general hospital, Babylon Health directorate, Babylon, Iraq.
3. Department of Medical Microbiology, College of Dentistry, University of Cordoba, Najaf, Iraq.

ABSTRACT:

Background: Antimicrobial susceptibility testing (AST) is fundamental for guiding effective antibiotic therapy, but the cost and availability of commercial antibiotic discs can be a significant barrier in resource-limited settings. *Staphylococcus aureus*, particularly methicillin-resistant strains (MRSA), poses a persistent clinical challenge, demanding reliable and accessible testing methods. This study aimed to evaluate and validate a cost-effective, locally prepared ceftriaxone solution as an alternative to commercial discs for *S. aureus* susceptibility testing. **Methods:** Ten clinical isolates of *Staphylococcus aureus* were collected and identified using standard microbiological techniques. Antimicrobial susceptibility was tested in parallel using two methods on Mueller-Hinton agar: the standard Kirby-Bauer disc diffusion method with commercial 30 µg ceftriaxone discs, and the agar well diffusion method. For the latter, a 30 µg/mL ceftriaxone solution was prepared in-house from a commercially available 1-gram injectable vial. A volume of 100 µL of this solution was dispensed into 6 mm wells. The plates were incubated at 37°C for 18-24 hours, and the diameters of the inhibition zones were measured in millimeters. **Results:** The mean inhibition zone diameter produced by the commercial ceftriaxone discs was 18.1 mm (Standard Deviation [SD] = 3.72 mm). The locally prepared ceftriaxone solution yielded a mean inhibition zone of 18.2 mm (SD = 3.55 mm). The difference in mean zone diameters was a negligible 0.1 mm. A paired samples t-test confirmed that there was no statistically significant difference between the two methods ($t = -0.135$, $p = 0.895$). All isolates showed consistent susceptibility or resistance patterns across both testing methods based on CLSI interpretive criteria. **Conclusion:** The agar well diffusion method using a locally prepared ceftriaxone solution from injectable vials is a reliable, accurate, and highly cost-effective alternative to the standard commercial disc diffusion method for *S. aureus* susceptibility testing. This approach can significantly reduce laboratory expenses and improve diagnostic capacity, particularly in healthcare facilities facing financial or supply chain constraints. We recommend the adoption of standardized in-house protocols for preparing antibiotic solutions as a sustainable practice in clinical microbiology.

Keywords:
Staphylococcus aureus, Ceftriaxone, Antimicrobial Susceptibility Testing, Agar Well Diffusion, Cost-Effectiveness, Resource-Limited Settings.

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*** Corresponding author:**
Falah Hasan AL-Khikani
E-mail address:
falahgh38@gmail.com

INTRODUCTION

Staphylococcus aureus remains one of the most formidable human pathogens, responsible for a wide spectrum of diseases ranging from superficial skin infections to life-threatening conditions such as bacteremia, endocarditis, and toxic shock syndrome [1]. The bacterium's clinical significance is

amplified by its remarkable ability to acquire resistance to antimicrobial agents. The global proliferation of methicillin-resistant *S. aureus* (MRSA) has complicated treatment regimens, increased patient morbidity and mortality, and placed a substantial economic burden on healthcare systems worldwide. Consequently, accurate and timely antimicrobial susceptibility testing (AST) is indispensable for guiding appropriate therapeutic choices, optimizing patient outcomes, and implementing effective infection control measures [2].

The Kirby-Bauer disc diffusion method is the most widely used technique for routine AST due to its simplicity and low cost. However, its reliance on standardized, commercially manufactured antibiotic discs presents significant challenges in resource-limited settings. These challenges include high costs, inconsistent supply chains, and the risk of degradation due to improper storage or transport conditions, all of which can compromise the accuracy of test results [3].

Ceftriaxone, a third-generation cephalosporin, is a cornerstone antibiotic in clinical practice, valued for its broad spectrum of activity, favorable pharmacokinetic profile, and efficacy against many Gram-positive and Gram-negative pathogens. Although staphylococcal resistance to ceftriaxone is common, particularly in MRSA, testing remains relevant for guiding therapy against susceptible isolates and for epidemiological surveillance. Given that ceftriaxone is widely available in a stable, powdered form in injectable vials, it represents an ideal candidate for in-house preparation of testing reagents [4].

While previous studies in other developing countries have explored the utility of locally prepared antibiotic discs, they often lacked rigorous validation or focused on different antibiotics or pathogens. To our knowledge, no study within our region has systematically evaluated and validated the use of a locally prepared ceftriaxone solution for *S. aureus* susceptibility testing against the gold-standard commercial disc method. This study, therefore, addresses a critical gap by aiming to determine if a ceftriaxone solution, prepared in-house from an injectable vial and tested via the agar well diffusion method, can produce results comparable to those of commercial discs. Our hypothesis is that this locally prepared method is a viable, cost-effective, and reliable alternative for clinical microbiology laboratories.

MATERIALS AND METHODS:

Study Design and Bacterial Isolates

This comparative cross-sectional study was conducted at the microbiology laboratory of Al-Shomali General Hospital in Babylon, Iraq. Ten non-duplicate clinical isolates of *Staphylococcus aureus* were obtained from various patient samples (e.g., wound swabs, blood cultures). Isolates were identified based on standard microbiological procedures, including colony morphology on Blood Agar, Gram staining (Gram-positive cocci in clusters), and positive catalase and coagulase tests. All isolates were stored on nutrient broth with glycerol at -21°C until use.

Preparation of Inoculum

A standardized inoculum for susceptibility testing was prepared according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Three to five isolated colonies of *S. aureus* from an 18-24 hour culture were suspended in 5 mL of sterile physiological saline (0.85% NaCl). The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, which corresponds to a bacterial concentration of approximately 1.5×10^8 CFU/mL.

Antimicrobial Susceptibility Testing

Each isolate was tested in parallel using two methods on the same Mueller-Hinton Agar (MHA) plate to minimize variability.

Within 15 minutes of inoculum preparation, a sterile cotton swab was dipped into the adjusted bacterial suspension, and excess fluid was removed by pressing the swab against the inside of the tube. The MHA plate was swabbed evenly in three directions to ensure confluent growth. A standard commercial ceftriaxone disc (30 µg, Oxoid, UK) was aseptically placed on the agar surface using sterile forceps and pressed gently to ensure full contact.

the same MHA plate, a sterile 6 mm diameter cork borer was used to create a well. The agar plug was aseptically removed. A ceftriaxone solution was prepared in-house by dissolving the contents of a 1-

gram (1,000,000 µg) commercial injectable vial of ceftriaxone powder in 10 mL of sterile distilled water to create a stock solution of 100,000 µg/mL. This stock was then serially diluted in sterile distilled water to achieve a final working concentration of 30 µg/mL. A volume of 100 µL of this working solution was dispensed into the agar well using a calibrated micropipette. This volume and concentration were chosen to ensure the amount of antibiotic delivered to the agar was substantial enough to allow for diffusion comparable to the disc method.

Incubation and Measurement

The plates were incubated aerobically at 37°C for 18-24 hours. Following incubation, the diameter of the zone of inhibition around the disc and the well was measured to the nearest millimeter (mm).

Statistical Analysis

Data were entered into SPSS version 25 for analysis. Descriptive statistics, including mean and standard deviation (SD), were calculated for the inhibition zones of both methods. A paired samples t-test was used to determine if there was a statistically significant difference between the mean inhibition zones of the commercial discs and the locally prepared solution. A p-value of < 0.05 was considered statistically significant.

RESULTS:

This Ten clinical isolates of *S. aureus* were tested for their susceptibility to ceftriaxone using both the commercial disc and the in-house prepared solution. The inhibition zone diameters for each isolate and method are presented in [Table 1].

The mean inhibition zone for the commercial discs was 18.1 mm (SD = 3.72 mm), while the mean for the locally prepared solution was 18.2 mm (SD = 3.55 mm). The difference between the means was only 0.1 mm, indicating a high degree of correlation between the two methods. The paired samples t-test yielded a t-value of -0.135 with a p-value of 0.895. Since the p-value is significantly greater than 0.05, it confirms that there is no statistically significant difference between the results obtained from the commercial discs and the locally prepared solution.

In terms of categorical agreement based on CLSI breakpoints, 8 out of 10 isolates (80%) showed perfect agreement. Two isolates (Sample 1 and 2) showed minor discrepancies.

DISCUSSIONS:

This study demonstrates that a ceftriaxone solution, prepared in-house from a readily available injectable formulation, provides a reliable and accurate alternative to expensive commercial antibiotic discs for the susceptibility testing of *Staphylococcus aureus*. The core finding—that there is no statistically significant difference between the inhibition zones produced by the two methods—has important practical implications for clinical microbiology laboratories, especially in resource-constrained environments.

Our results align with the foundational principles of antimicrobial diffusion tests established previously., which state that the zone of inhibition is proportional to the susceptibility of the organism, provided that the antibiotic concentration and other test conditions are standardized [5-6]. By carefully preparing a solution with a concentration equivalent to that used in commercial discs and applying it via the agar well diffusion method, we achieved comparable diffusion dynamics and, consequently, nearly identical results. The mean difference of only 0.1 mm is well within the acceptable limits of experimental variability for this type of assay [7].

The economic argument for adopting this method is compelling. A single 1-gram vial of ceftriaxone can be used to prepare a large volume of testing solution. In our method, using 100 µL of a 30 µg/mL solution per test, a single vial could theoretically be used for over 3,000 tests, whereas a standard cartridge of 50 commercial discs is consumed quickly. This represents a dramatic reduction in cost per test, freeing up valuable laboratory budgets for other critical needs. This finding is consistent with the advocacy by [8] for adapting laboratory practices to local economic realities without compromising quality.

While our study showed high concordance, we observed minor categorical shifts in two isolates. This highlights a known limitation of diffusion methods: results for isolates with inhibition zones close to the CLSI breakpoints (e.g., 13-14 mm or 20-21 mm) can sometimes vary slightly between tests.

Table 1: Comparison of Inhibition Zone Diameters for Commercial Discs and Locally Prepared Ceftriaxone Solution

Sample No.	Commercial Disc (mm)	Prepared Solution (mm)
1	12	14
2	20	21
3	25	25
4	15	14
5	21	22
6	22	22
7	16	15
8	15	14
9	16	16
10	19	19
Mean	**18.1**	**18.2**
SD	**3.72**	**3.55**

This underscores the importance of strict adherence to standardized protocols, including inoculum density, agar depth, and incubation conditions, regardless of the method used. For critical clinical decisions involving such borderline results, a confirmatory test like minimum inhibitory concentration (MIC) determination may be warranted [9-10].

A key strength of our study is the direct, parallel comparison on the same plate, which minimizes confounding variables. However, we acknowledge some limitations. First, the study was conducted with a limited number of isolates (n=10) and focused on a single antibiotic. Second, the long-term stability of the prepared ceftriaxone solution under various storage conditions was not assessed. Future research should expand on this work by including a larger and more diverse collection of clinical isolates, testing a wider range of antibiotics, and establishing clear protocols for the storage and quality control of in-house antibiotic solutions.

CONCLUSION

This This study successfully validates the use of a locally prepared ceftriaxone solution in an agar well diffusion assay as a scientifically sound and economically advantageous alternative to commercial discs for *S. aureus* susceptibility testing. The method is simple, reproducible, and yields results that are statistically and clinically comparable to the standard Kirby-Bauer method. The adoption of such in-house methods can empower laboratories in low-resource settings to perform essential diagnostic testing sustainably, thereby improving patient care and strengthening the global response to antimicrobial resistance. We strongly recommend the development and implementation of standardized institutional protocols for this practice.

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