Case Series

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To determine Minimum Inhibitory Concentration (MIC) of Cefixime in Mahacef® Tablet 200mg and API Cefixime trihydrate compacted IP (API)

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Abstract

Background: Antibiotic resistance is rising to dangerously high levels globally. New resistance mechanisms are emerging and spreading, thereby threatening our ability to treat common infectious diseases. In places where antibiotics can be bought for human or animal use without a prescription, the emergence and spread of resistance is made worse. This study endeavours to determine minimum inhibitory concentration (MIC) of Mahacef® 200mg Tablet and Cefixime Trihydrate API, both supplied by Mankind Pharma Limited. **Methodology:** Antibacterial activity of Cefixime on Salmonella enterica subsp. enterica serover Typhimurium was determined by Agar Dilution Method with Mueller Hinton Agar. Available strain of Salmonella enterica subsp. enterica serover Typhimurium (ATCC® 14028™) was used with Inoculum size was approximately 1000 cfu / plate. Dilutions were prepared in Phosphate buffer (pH 7.0, 0.1 mol/L) for Mahacef® 200 mg Tablet and Cefixime Trihydrate API, and used for the study. **Results:** Findings of this study demonstrate that both Mahacef® 200mg Tablet and Cefixime Trihydrate API have shown excellent antibacterial activity in vitro. MICs for the drug product as well as for the drug substance (API) are found to be 0.1 µg/mL against Salmonella enterica subsp. enterica serover Typhimurium. **Conclusion:** Mahacef® 200mg andCefixime Trihydrate API clearly establish support for its antimicrobial effectiveness and therefore, support values determined MIC for the viewpoint of medical fraternity that Cefixime is effective for management of typhoid fever.

Keywords: Minimum Inhibitory Concentration; Cefixime; Cefixime trihydrate compacted IP; Antibiotic resistance.

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Introduction

In vitro antibacterial activity of Cefixime in Mahacef® Tablet 200mg and API Cefixime trihydrate compacted IP (API) was evaluated.

Using a conventional Agar Dilution method, the Minimum Inhibitory Concentration (MIC) of the antibiotic was determined against Salmonella enterica subsp. enterica serover Typhimurium. The MIC of Cefixime was found to be 0.1 µg/mL.

Outcome of the study is clinically meaningful since MIC for Cefixime is well below value reported for the antibiotic against Salmonella species, and therefore, supports the viewpoint of medical fraternity that Cefixime is effective for management of typhoid fever.[1]

Antibiotic resistance is rising to dangerously high levels globally. New resistance mechanisms are emerging and spreading, thereby threatening our ability to treat common infectious diseases.

In places where antibiotics can be bought for human or animal use without a prescription, the emergence and spread of resistance is made worse. Similarly, in countries without standard treatment guidelines, antibiotics are often over-prescribed by health workers and veterinarians and over-used by the public.

In a Joint News Release (dated 29 April 2019), the United Nations, international agencies and experts have demanded immediate, coordinated and ambitious action to avert a

potentially disastrous drug-resistance crisis. The Release goes on to state that "if no action is taken, drug-resistant diseases could cause 10 million deaths each year by 2050 and damage economy as fatal as 2008-2009 global financial crisis". In fact, the estimate is that by 2030, up to 24 million people could face extreme poverty due to antimicrobial resistance. In India alone, multi-drug resistant organisms are responsible for over 58,000 infant deaths each year.[2]

Salmonella is an infamous pathogen that causes gastroenteritis and typhoid fever in humans, and 94 million cases of salmonellosis are globally reported every year. Salmonella infection is mainly caused by foods (meat, eggs, fish, and shellfish) with symptoms mainly that include nausea, vomiting, abdominal pain, diarrhea, and fever.[2]

S. Typhimurium infects a wide range of mammals and birds in addition to being a leading cause of foodborne gastroenteritis in human populations. Therefore, control of S. Typhimurium infection in livestock is of great economic importance which is closely related to human food chain, particularly in swine and cattle. Additionally, S. Typhimurium causes an invasive disease in mice, which has been used extensively as a model for pathogenicity and human typhoid fever.[3]

Various serotypes (approx. 2,500) of Salmonella spp. have been reported. Serotypes serve as epidemiological markers, and specific Salmonella spp. serotypes are associated with human disease. In early 1990s, S. Typhi was frequently detected in Korea, and S. Enteritidis and S. Typhimurium were frequently observed which are also related to human infections.[4]

It is important therefore, to use antibiotics in true bacterial infections and in such doses so as to increase the likelihood of therapeutic effectiveness. Inefficiency of medical therapies used in order to cure patients with bacterial infections requires not only to actively look for new therapeutic strategies but also to carefully select antibiotics based on variety of parameters, including microbiological. Among such microbiological parameters is the minimum inhibitory concentration (MIC) of the antimicrobial.[5]

Cefixime is a semisynthetic, 3rd generation cephalosporin antibacterial for oral administration. Cefixime tablets and suspension, given orally, are about 40% to 50% absorbed whether administered with or without food; however, time to maximal absorption is increased approximately 0.8 hours when administered with food. A single 200 mg tablet of cefixime produces an average peak serum concentration of approximately 2 mcg/mL (range 1 to 4 mcg/mL). Peak serum concentrations occur between 2 and 6 hours following oral administration of a single 200 mg tablet, a single 400 mg tablet or 400 mg of cefixime suspension. As with other cephalosporins, the bactericidal action of cefixime results from inhibition of cell wall synthesis. Cefixime is stable in the presence of certain beta-lactamase enzymes. As a result, certain organisms resistant to penicillins and some cephalosporins due to the presence of beta-lactamases may be susceptible to cefixime. Cefixime exhibits in vitro MICs of 1.0 µg/mL or less against most (≥ 90%) species (MIC90 of 0.21 µg/mL against Salmonella species).[1,6,7]

This study endeavors to determine minimum inhibitory concentration (MIC) of Mahacef® 200mg Tablet and Cefixime Trihydrate API, both supplied by Mankind Pharma Limited**.**

Material & Method

Description of Methodology

Standardized methods are essential for susceptibility testing. Methods ought to be highly sensitive to variations in several factors, for example, size of inoculum, contents and acidity of the growth medium, time and temperature of incubation. Among different methods used for determination of MIC, Agar Dilution is regarded as the "gold standard" for susceptibility testing. Accordingly, this method was used in current study.

Selection of organism

Ki-Bok Yoon and co-workers studied Antibiotic Resistance Patterns and Serotypes of Salmonella spp. A total of 276 (a total of 22 different serotypes were divided among 276 Salmonella spp.) stocked Salmonella spp. were tested against following antibiotics: ampicillin, amoxicillin/clavulanic acid, ampicillin/sulbactam, cefalothin, cefazolin, cefotetan, cefoxitin, cefotaxime, ceftriaxone, imipenem, amikacin, gentamycin, nalidixic acid, ciprofloxacin, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole. Overall, it was found that S. Enteritidis and S. Typhimurium showed higher antibiotic resistance than the other Salmonella serotypes such as S. typhi, S. thompson etc. tested in that study.[4]

Accordingly, Salmonella enterica subsp. enterica serover Typhimurium was identified as the model organism for this study.

Materials

Mahacef® 200mg Tablet and Cefixime Trihydrate API were supplied by Mankind Pharma Limited, India. Apart from these Phosphate buffer, pH 7.0, 0.1mol/mL, sufficient quantity of Mueler Hinton agar, Salmonella strains, petri dishes, calibrated graduated pipettes, disposable loops etc. were used for the study. Following are details of Mahacef® 200mg Tablet and Cefixime Trihydrate API which were used for the study.

Table 1: Details of Mahacef® and Cefixime Trihydrate API

Material	Batch number	Manufacturing date	Expiry date
Cefixime Tablets IP (Mahacef® 200)	C0AKU016	03/2021	02/2023
Cefixime Trihydrate API	CFEC210329	06/2021	05/2024
Mueller Hinton Agar (MHA)	1292417	-	30/09/2025

Methods

Antibacterial activity of Cefixime on Salmonella enterica subsp. enterica serover Typhimurium was determined by Agar Dilution Method with Mueller Hinton Agar. Available strain of Salmonella enterica subsp. enterica serover Typhimurium (ATCC® 14028™) was used with Inoculum

size was approximately 1000 cfu / plate.

Dilutions were prepared in Phosphate buffer (pH 7.0, 0.1 mol/L) as per Table 2 below for Mahacef® 200mg Tablet and Cefixime Trihydrate API, and used for the study:

Table 2: Dilution in Phosphate Buffer

Sample ID	1	2	3	4	5	6	7	8	9	10
Final Concentration (µg/mL)	0.03	0.05	0.1	0.2	0.4	0.5	0.6	0.8	1	2

MH agar medium was prepared and sufficient quantity was poured in the petri dishes. Petri dishes were prepared in triplicate for each concentration of Measurements were carried out in triplicate at each concentration level of the antibiotic.

Inoculum was spread on prepared petri dishes and incubated at 35°C ± 2°C for approx. 18 hrs. The petri dishes were observed for colonies. Results are provided in Table 3 and Table 4 for Mahacef® 200mg Tablet and Cefixime Trihydrate API.

Results

Following results were observed for Mahacef® 200mg Tablet

Table 3: Test Results of Mahacef® 200mg Tablet

S. No	Final Concentration (µg/mL)	Colonies obtained on MHA plate		
		Plate-1 (cfu)	Plate-2 (cfu)	Plate-3 (cfu)
1	0.03	410	430	470
2	0.05	30	34	40
3	0.1	Nil	Nil	Nil
4	0.2	Nil	Nil	Nil
5	0.4	Nil	Nil	Nil
6	0.5	Nil	Nil	Nil
7	0.6	Nil	Nil	Nil
8	0.8	Nil	Nil	Nil
9	1.0	Nil	Nil	Nil
10	2.0	Nil	Nil	Nil
11	Blank	Nil		

MIC was observed to be 0.1 µg/mL of Cefixime

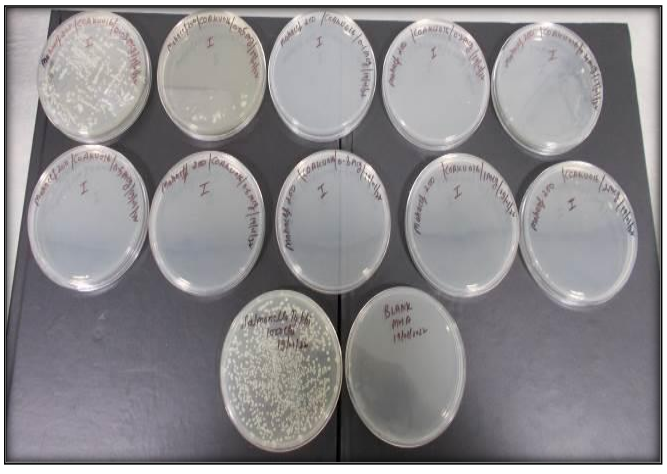
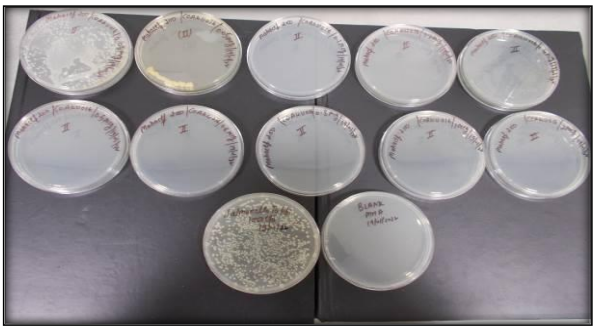


Plate-1



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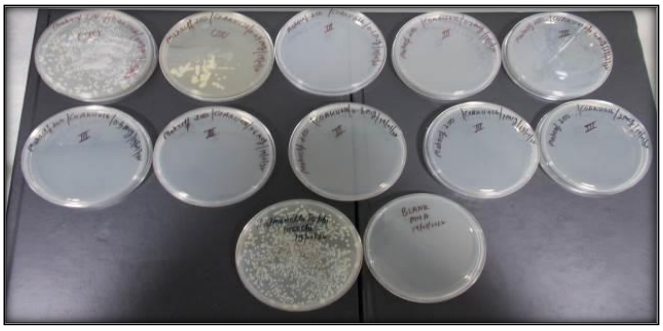

Plate-3

Figure 1: Microbial Growths in Presence (Cefixime for Mahacef® 200mg Tablet)

Following results were observed for Cefixime Trihydrate API

Table 1: Test results of Cefixime Trihydrate API

S. No	Final Concentration (µg/mL)	Colonies obtained on MHA plate		
		Plate-1 (cfu)	Plate-2 (cfu)	Plate-3 (cfu)
1	0.03	519	548	525
2	0.05	42	29	45
3	0.1	Nil	Nil	Nil
4	0.2	Nil	Nil	Nil
5	0.4	Nil	Nil	Nil
6	0.5	Nil	Nil	Nil
7	0.6	Nil	Nil	Nil
8	0.8	Nil	Nil	Nil
9	1.0	Nil	Nil	Nil
10	2.0	Nil	Nil	Nil
11	Blank	Nil		

MIC was observed to be 0.1 µg/mL of Cefixime.

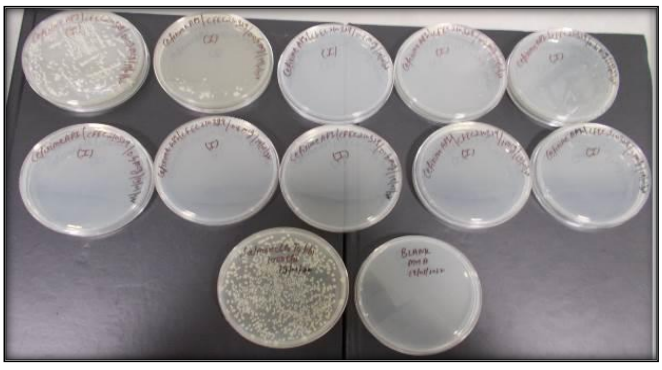

Plate-1

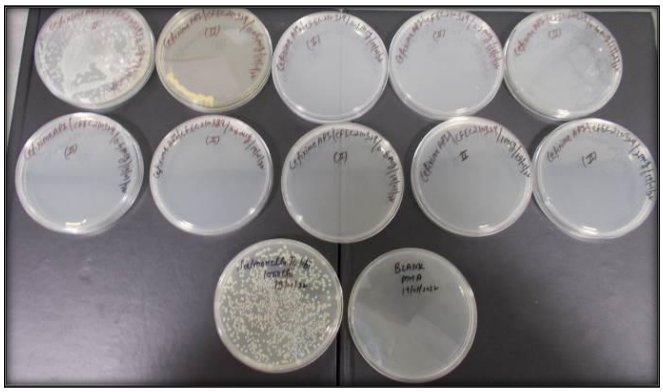

Plate-2

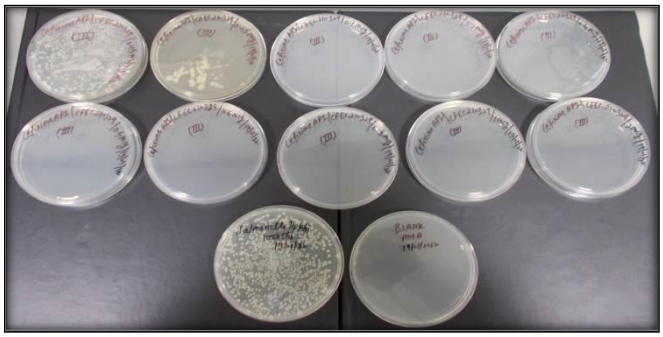

Plate-3

Figure 2: Microbial Growths in Presence of Cefixime (Cefixime Trihydrate API)

Discussion

As with other cephalosporins, the bactericidal action of cefixime results from inhibition of cell wall synthesis. Cefixime is stable in the presence of certain beta-lactamase enzymes. As a result, certain organisms resistant to penicillins and some cephalosporins due to the presence of beta-lactamases may be susceptible to cefixime.

Cefixime, a 3rd generation oral cephalosporin is commercially available, and its efficacy and safety in children have been well proven by various clinical trials and post marketing surveillance studies. Several clinical trials have also shown its usefulness in the treatment of paediatric typhoid fever. According to Matsumoto et al., the new antibiotics, cefixime, cefdinir, ceftriaxone, ofloxacin, and ciprofloxacin, showed excellent antibacterial activities against the clinical isolates of S. typhi. This supports clinical usefulness of these new antibiotics for treatment of typhoid fever. These antibiotics have already been proven to be clinically effective. Quinolones or 3rd generation cephalosporins have been recommended to treat suspected typhoid fever in areas where Multi Drug Resistant S. typhi is prevalent, until culture and sensitivity results are available. Cefixime provides a safe, effective and cheaper oral option for the treatment of typhoid fever in children, especially in endemic MDR areas.[8]

Findings of this study demonstrate that both Mahacef® 200mg Tablet and Cefixime Trihydrate API have shown excellent antibacterial activity in vitro. MICs for the drug product as well as for the drug substance (API) are found to be 0.1 µg/mL against Salmonella enterica subsp. enterica serover Typhimurium.

This value of MIC is in line with values defined in literature (in vitro MICs of 1.0 µg/mL or less is reported against most (≥ 90%) species, with MIC90 of 0.21 µg/mL against Salmonella species).[1,6,7]

As a tie-in of MIC findings of this study to clinical relevance, it is reported that Cefixime produces average peak serum concentrations of approximately 2 mcg/mL (range 1 to 4 mcg/mL) after administration of single tablet of 200mg which is well above the MIC observed for Mahacef®.

Furthermore, an established approach based on Time above MIC (T>MIC) was also evaluated. T>MIC is an accurate and consistent predictor of antimicrobial activity, derived in consideration of relevant pharmacokinetic parameters (i.e., Cmax, Tmax, T1/2). Pharmacokinetic parameters for Cefixime for deriving T>MIC were obtained from information in public domain. Thereafter, this parameter was estimated for Mahacef® 200 mg as tabulated below (Table 5).[9]

Table 5: Estimated Time above MIC Values

Product	T>MIC (Hr)	T>MIC (%)
Mahacef® 200mg	20.35	84.80

Note: Coverage (i.e. T>MIC, in %) depicts time duration for which drug levels in plasma are above MIC during the dosing interval of 24 hours.

Despite global surge of antimicrobial resistance among Salmonella enterica clinical isolates, the MIC values from this study support the viewpoint that level of drug resistance to Cefixime is not high.

S. Typhimurium being more resistant than S. typhi, S. thompson etc. against 3rd generation cephalosporin such as cefotaxime, ceftriaxone, it is expected that Mahacef® 200mg Tablet which contains Cefixime will also be effective against S. typhi.

Conclusion

Mahacef® 200mg and Cefixime Trihydrate API clearly establish support for its antimicrobial effectiveness and therefore, support values determined MIC for the viewpoint of medical fraternity that Cefixime is effective for management of typhoid fever.

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