Original Article

Determination of Minimum Inhibitory Concentration (MIC) of Tigecycline against Salmonella typhi

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Abstract:

Introduction: Typhoid fever, caused by S. Typhi, is prevalent in developing countries, particularly the Indian subcontinent. Salmonella infections can cause enteric fever, gastroenteritis, septicemia, and non-typhoidal Salmonellae (NTS) infections, especially in immunocompromised patients.

Objective: To determine the Minimum Inhibitory Concentration (MIC) of Tigecycline against Salmonella Typhi.

Methodology: This interventional study conducted at Department of Pharmacology & Therapeutics in collaboration with Department of Microbiology at Ad-din Sakina Women's Medical College, Jashore during March 2023 to April 2023.MIC of Tigecycline was determined by Broth Dilution Technique against standard strain of Salmonella typhi ATCC 24683.

Result: The MIC of Tigecycline against Salmonella typhi was 2.0 μg/ml.

Conclusion: Tigecycline is a potential therapeutic agent for Salmonella typhi infection, and should be restricted on the basis of blood culture and in MDR and XDR cases of typhoid fever only.

Key Words: Salmonella, Tigecycline, Minimum Inhibitory Concentration

Introduction:

Typhoid fever which is caused by S. Typhi is endemic in developing countries; more so in the Indian subcontinent.[1] Infections with Salmonellae can result in various clinical presentations like enteric fever, gastroenteritis, septicemia with or without supportive lesion and carrier state. Salmonella typhi and paratyphi A, B and C cause typhoid fever and paratyphoid fever respectively, while non typhoidal Salmonellae (NTS) that has more than 2500 serotypes, causes gastroenteritis and invasive infections like meningitis and osteomyelitis in immunocompromised patients adults and children.[2] Salmonella typhi is mostly acquired directly or indirectly through human feces by fecal-oral route from the diseased person or a carrier.

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Salmonella infections, especially those involving the blood stream, have a high mortality rate (about 30%). This can be reduced to about 1% with appropriate use of antibiotics.[3, 4] However, resistance of Salmonella Typhi to chloramphenicol, cotrimoxazole and ampicillin developed in the 1980s. Threat of growing resistance to antibiotics is of grave concern to human health as it can lead to prolonged illness and more rate of complications.[5]

The World Health Organization (WHO) recommends treatment with azithromycin, ciprofloxacin, or ceftriaxone due to widespread resistance to older first-line antimicrobials. With an increasing use of fluoroquinolones against enteric fever, gradually resistance also developed ciprofloxacin. Resistance to third generation cephalosporins such as ceftriaxone is beginning to emerge as well.[6] Decades of empiric antibiotic use have resulted in the development of these MDR organisms (resistant to ampicillin, co-trimoxazole, and chloramphenicol) followed by extensively drug-resistant (XDR) S. typhi strains (resistant to chloramphenicol, ampicillin, co-trimoxazole, fluoroquinolones, and ceftriaxone).

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An outbreak of MDR S. Typhi in late 1990s in Tajikistan caused more than 24,000 infections.[7] The world witnessed its first case of Extensively Drug-Resistant (XDR) Typhoid Fever in 2016 in Pakistan.[8] More recently, the World Health Organization (WHO) recorded that there were 5,274 cases of XDR typhoid fever out of a total of 8,188 cases of typhoid fever reported in Pakistan from November 2016 up to December 2018. [9]

Similar outbreaks have been documented worldwide, particularly in regions such as Southeast Asia, the Indian subcontinent, Africa, and South America [10,11]. This highlights the escalating challenge of drug-resistant typhoid infections globally. The emergence of resistance has significantly reduced the available therapeutic options for treating typhoid and other Salmonella infections. Managing outbreaks of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Salmonella Typhi poses significant challenges, particularly in developing countries with limited resources. Therefore, there is an urgent need to explore new approaches for treating drug-resistant Salmonella strains.

One promising avenue in the treatment of typhoid is the antibiotic tigecycline, which is not commonly used for Salmonella infections. Tigecycline belongs to the glycylcycline class of antibiotics and shares structural similarities with tetracycline antibiotics. It exhibits a broad spectrum of activity, effectively targeting a wide range of gram-positive, gram-negative, and anaerobic bacteria. Its mechanism of action involves binding to the 30S ribosomal subunit in susceptible bacteria, ultimately hindering protein synthesis by impeding the incorporation of amino acids into peptide chains, thereby halting bacterial growth.[12]

The objective of this study was to determine the minimum inhibitory concentration (MIC) of Tigecycline against Salmonella typhi. MIC represents the lowest concentration of a drug required to prevent visible in-vitro growth of the organism. This research seeks to shed light on the effectiveness of Tigecycline as a potential treatment option against drug-resistant Salmonella typhi strains, in the face of rising antibiotic resistance.

Materials and method:

The interventional study was conducted in the Department of Pharmacology and Therapeutics in collaboration with the Department of Microbiology at Ad-din Sakina Women's Medical College, Jashore, Bangladesh during the period of March to April 2023.

Ethical Approval:

Ethical clearance was obtained from Ethical Review Committee (ERC) and Institutional Review Board (IRB) of Ad-din Sakina Women's Medical College (ASWMC), Jashore.

Collection of antibiotic Tigecycline:

Tigecycline antibiotics for this study were obtained through the purchase of Injection Tegalon vials (500 mg) from the local market. These vials were manufactured by Healthcare Pharmaceuticals LTD, Bangladesh.

Test organism:

Standard reference strain of Salmonella typhi, ATCC 24683 was collected from the Department of Microbiology of Ad-din Sakina Women's Medical College, Jashore.

Procedure of Experiment:

Determination of MIC of Tigecycline against test organisms

Technique: Broth dilution.

Preparation of stock solution of Tigecycline:

Five hundred (500) mg of Tigecycline powder was mixed well with 500 ml of sterile Distilled Water (DW) by using a sterile syringe. The prepared Tigecycline Injection had the concentration of 500 mg in 500 ml. So, 1 ml solution contain 1 mg Tigecycline (Stock Tigecycline solution-I). Then 1 ml of stock Tigycycline solution-I was mixed with 99 ml of sterile D/W. This 1:100 dilution of stock Tigycycline solution-I had the concentration of 10 μg/ml. This solution was marked as Stock Tigecycline Solution-II which was used as stock solution for the determination of MIC of Tigecycline.

Calculations:

Tigecycline 500 mg + 500 ml D/W.

So, 500 mg Tigecycline in 500 ml

Thus 1 ml contains 1 mg of Tigecycline (Stock Tigecycline Solution-I)

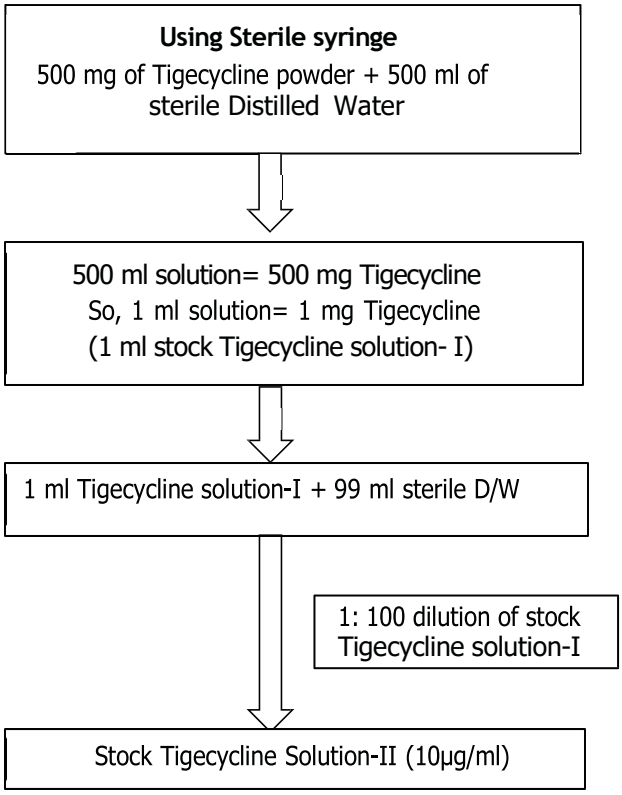
1 ml of solution + 99 ml D/W (1:100 dilution).

So, 100 ml contains 1 mg Tigecycline = 1000 μg Tigecycline

So, in 1 ml, the concentration is 1000/100 = 10 μg Tigecycline /ml (Stock Tigecycline Solution-II)

This stock Tigecycline solution-II (concentration 10 μg/ml) was used for the determination MIC of Tigecycline by broth dilution technique

Flowchart:



Preparation of different concentrations of Tigecycline solution:

Set - I: Tigecycline solution was made by adding 0.25 ml of stock Tigecycline solution-II with 9.75 ml of Trypticase soya broth medium. The concentration of Tigecycline in this dilution was 0.25 µg/ml.

Calculation:

1 ml of stock Tigecycline solution contains 10 µg of Tigecycline. (Stock Tigecycline Solution-II)

So, 0.25 ml Tigecycline solution contains 2.5 µg of Tigecycline

So 10 ml of set I preparation contains 2.5 µg of Tigecycline

And thus 1 ml of set I preparation contains 25 µg of Tigecycline

Set - II: Tigecycline solution was made by adding 0.5 ml of stock Tigecycline solution-II with 9.5 ml of Trypticase soya broth medium. The concentration of Tigecycline in this dilution was 0.5 µg/ml.

Similarly, **Set-III, IV, V and VI** of Tigecycline solution respectively were made by adding a measured amount of stock Tigecycline solution-II with the measured amount of broth medium. The concentrations of Tigecycline were 0.75 µg/ml, 1 µg/ml, 1.5 µg/ml and 2 µg/ml respectively: (Table 1).

Control - 1: was made with 10 m1 of Trypticase soya broth medium (to be inoculated with bacterial suspension) in test tubes.

Control - 2: was made with 10 m1 of Trypticase soya broth medium (no inoculation with bacterial suspension) in test tubes. (Table 1) With each 10 ml preparation except control-1 (set VII) 20 µl bacterial suspensions were added after matching its opacity with that of 0.5 McFarland Standard.

Table-I

Composition and different concentrations of working Tigecycline solutions and the controls:

No. of Sets	Stock Tigecycline solution-II (ml)	Trypticase soya Broth media (ml)	Total (ml)	Concentration of Tigecycline (µg/ ml)	Test organism (µl)
I	0.25	9.75	10	0.25	20
II	0.5	9.50	10	0.5	20
III	0.75	9.25	10	0.75	20
IV	1	9	10	1	20
V	1.5	8.5	10	1.5	20
VI	2	8	10	2	20
VII	Control-1	10	10	-	-
VIII	Control-2	10	10	-	20

Inoculation of bacterial suspension to different concentrations of stock Tigecycline in test tubes:

After matching the turbidity of bacterial suspension with 0.5 McFarland standards, 20 μl or one drop (0.02 m1) of bacterial suspension of Salmonella typhi is inoculated. These inoculums were also added to the control -2 but were not added to Control-1.

Incubation: The test tubes were marked set wise with black marker and were placed in the incubator at 37° C for 18 -24 hours.

Examinations of test organisms in different dilutions and concentrations of Tigecycline: After 18 to 24 hours of incubation at 37° C, the growth of test organisms in each preparation of Tigecycline was examined and compared against that of control by matching their turbidity. The clear preparations were considered as no growth of bacteria and turbid one as growth of bacteria. The MIC was reported as the lowest concentration of Tigecycline required to prevent the visible growth of test organisms. The observations and results of the experiment were shown in Table-II.

Subculture of materials from effective dilutions of Tigecycline in MacConkey agar media: The materials from last two sets of growth and all sets of no growth of Tigecycline preparations were subcultured in the pure MacConkey (solid) media plates (without antibiotic and antibiotic mixed media). After 18 to 24 hours of incubation at 37°C, the growth of test organisms were examined.

Observations and results:

Table-II shows visible growth of Salmonella typhi observed at Set-I to Set-V. But the organisms failed to grow at Set-VI. So the minimum inhibitory concentration (MIC) of Tigecycline against Salmonella typhi was 2.0 μg/ml.

Table-II

MIC of Tigecycline against Salmonella typhi

No of Sets	Concentration (μg/ ml)	Salmonella typhi
Set-I	0.25	Growth
Set-II	0.5	Growth
Set-III	0.75	Growth
Set-IV	1	Growth
Set-V	1.5	Growth
Set-VI	2	No Growth
Set-VII	Control-1(Trypticase soya broth + No bacteria inoculation)	No Growth
Set-VIII	Control-2 (Trypticase soya broth+ Bacterial inoculation with no antibiotic)	Growth

Table-II also showed control-1 containing Trypticase soya broth medium without any bacterial inoculum had no visible growth and control -2 containing Trypticase soya broth medium with bacterial inoculum observed their visible growth.

Result of Experiment: The MIC of Tigecycline against Salmonella typhi was 2.0 μg/ml at set VI.

Discussion:

Typhoid fever which is caused by S. Typhi is endemic in developing countries; more so in the Indian subcontinent.[1] Salmonella infections, especially those involving the blood stream, have a high mortality rate (about 30%). This can be reduced to about 1% with appropriate use of antibiotics.[3, 4] Threat of growing resistance to antibiotics is of grave concern to human health as it can lead to prolonged illness and more rate of complications.[5] Outbreaks of MDR- and XDR- Typhoid fever have been documented worldwide, particularly in regions such as Southeast Asia, the Indian subcontinent, Africa, and South America.[10, 11] The emergence of resistance has significantly reduced the available therapeutic options for treating typhoid and other Salmonella infections. Therefore, there is an urgent need to explore new approaches for treating drug-resistant Salmonella strains.

The objective of this study was to determine the minimum inhibitory concentration (MIC) of Tigecycline against Salmonella typhi.

The study was conducted during the period of March 2023 to April 2023 in the department of Pharmacology and Therapeutics with the collaboration of Department of Microbiology, Ad-din Sakina Women's Medical College, Jashore to determine the MIC of antibiotic Tigecycline against standard strain of Salmonella typhi. It was an interventional study. The MIC of antibiotic Tigecycline was determined by broth dilution technique. The stock solution of Tigecycline was made. Then the working solution of various concentrations was made by diluting the stock Tigecycline solution. The concentrations were 0.25 μg/ ml,. 5 μg/ ml, and 0.75 μg/ml, 1 μg/ml, 1.5 μg/ml, and 2 μg/ml. The MIC of Tigecycline against Salmonella typhi was 2.0μg/ml. A near similar type of study was done at Department of Microbiology, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India where the investigators found the MIC of Tigecycline against

Salmonella typhi was 2.0 μg/ml [13] which is similar to our study. Another study was done by Thomas R. Fritsche et al. in the year 2005 where the MIC of Tigecycline was determined against various species of Enterobacteriaceae including Salmonella spp where the MICs varies 2-8 μg/ml in different species.[14] From the study it is evident that the minimum inhibitory concentration of Tigecycline, **i.e.** 2.0μg/ml, is much lower than other Salmonella sensitive antibiotics like Ciprofloxacin and Azithromycin.[15]

Conclusion:

It is evident that the minimum inhibitory concentration of Tigecycline, i.e. 2.0μg/ml, is much lower than other Salmonella sensitive antibiotics like Ciprofloxacin and Azithromycin. But indiscriminate use of this antibiotic will cause antibiotic resistance. As Tigecycline is a potential therapeutic agent, its use should be restricted on the basis of blood culture and in MDR cases of typhoid fever only.

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