Original Article

Pharmacodynamics Analysis Of Ceftriaxone Against Klebsiella Pneumonia Isolated From Covid-19 Patients

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Abstract

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. Pneumonia is a general term for infections of the lungs that lead to inflammation of one or both lungs' parenchyma that is more often due to accumulation of fluids and white blood cells in the alveoli. It is the main cause of morbidity and mortality in human and animals. Pneumonia is usually bacterial in nature and due to the high risk of morbidity; the major causes of Pneumonia are S. pneumonia, K. pneumonia, S.aureus and other type of bacteria. The major health problems caused by K. pneumonia are considered as a common. ceftriaxone is a third generation of cephalosporin are widely used to treat serious infections caused by multi-resistant Gram-negative bacteria, however, beginning with the initial description to get rid of Pneumonia. The prevalence of K. pneumonia is 32% of all other causes of pneumonia. MIC recording 128 µg/ml that inhibited growth of K. pneumonia (256 µg/ml) is killed K. pneumonia so considers the (MBC), Time killing curve of each 2x MICs, and 4x MICs concentrations of ceftriaxone showed a distinguished bactericidal effect at 6th and 24hr respectively. The time of post-antibiotic effect that recorded in 1x MIC and 10x MIC culture after removal of Ceftrixone effect were 0.51 hr. (30.6 min) and 0.80 hr. (48 min.). MPC/MIC ratio is 2 for ceftriaxone.

Keywords: Ceftriaxone, Minimum Inhibitory Concentration, Mutant Prevention concentration, Post antibacterial effect, Time killing curve.

1.Introduction:

Coronavirus (COVID-19) is suspected to originate from an animal host (zoonotic) followed by a human to human transmission, Coronavirus associated with severe respiratory disease in humans emerged in the Middle East, the evolution of the Corona virus from 2003, 2013 to 2021 which has become the most deadly peak in humans, the virus evolved from the bat effect on Humans. (Al-Ajeeli, 2017; AL-Mutar, 2020). Pulmonary diseases of viral origin are often followed by the manifestation of secondary bacterial infections (Manohar et al., 2020).

Pneumonia is usually bacterial in nature and due to the high risk of morbidity, the major health problems caused Streptococcus pneumoniae, Klebsiella pneumonia, Staphylococcus aureus, Haemophilus influenzae type b, as well as the atypical pathogens Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella species. Today K. pneumoniae are considered as a common in human and animals the main causative organisms include (Liu et al., 2016).

Klebsiella pneumoniae belongs to the Enterobacteriaceae family and is described as a gram-negative, encapsulate, and non-motile bacterium. Virulence of the bacterium is provided by a wide array of factors that can lead to infection and antibiotic resistance (Jondle et al., 2018).

K.pneumoniae characteristically produce a β-lactamase so recommendations uses a broad-spectrum beta-lactam antibiotic (e.g. ceftriaxone, cefotaxim, piperacillin/tazobactam, ertapenem, or meropenem) in some time combination with a macrolide or fluoroquinolone is usually administered empirically to patients (Frei et al., 2010).

Ceftriaxone are semisynthetic cephalosporins antibacterial agents derived from cephalosporin-C, which is produced by cephalosporium acremonium. (Tucaliuc et al., 2019).

Ceftriaxone sodium is a third-generation cephalosporin (Khan et al., 2017). This agent is used for the treatment of various community-acquired infections and is also used for the treatment of infections with Neisseria gonorrhoeae and Salmonella typhi and pneumonia infection (Khan et al., 2017).

2.Material and methods

2.1. Bacterial Isolates and Identification:

Fifty samples of swab and sputum were taken from patients suffering from respiratory infection was obtained from the Medical isolation units in Al-Humeiyat and Al-Ramadi Teaching Hospital for Women and Children were collecting from 12/2020 – 2/2021.

Vitek® 2 System for Identification It was done in laboratory Al-Ramadi Teaching Hospital for Women and Children for identification of K.pneumoniae. by Biochemical tests and identification cards by gram-negative bacilli card (GNB) to read the kinetic fluorescence measurements, results were reported within 5 hrs (Nimer et al., 2016).

2.2. Pharmacodynamics Analysis

2.2.1. Estimation of Minimum Inhibitory Concentration (MIC)

According to Veiga et al., 2019 the microdilution tests were performed in sterile U-shape -bottomed 96-well microplates 100 μL of broth (Mueller Hinton broth) were added in all the wells of the plates. In the first well of each column (columns 1 to 11) was added 100 μL of antimicrobial solutions (serial dilutions were performed by passing 100 μL of wells 1 to 10 of the lines and 10 μL of the respective standardized inoculum was added (1.5×10^8 cfu/ml of K.pneumoniae). For the negative control left without bacterial the blank wells 200 μL of Mueller Hinton was added.

The plates were incubated at 35 °C for 22 h. After incubation, 20 μL of TTC solution 0.125% (w/v) was added into each well, and the plates were incubated again for 2 h.

2.3.2. Bacterial Time kill curve

The time-kill curve assay of ceftriaxone against Klebsiella pneumonia isolate were based on the National Committee for Clinical Laboratory Standards (NCCLS., 1999). ceftriaxone had been dissolved in Mueller-Hinton broth to prepare 10 mg/ml stock solution, after that, ceftriaxone concentrations from 4x MIC to 0.25x MIC had been prepared .Bacterial colonies were calculated at 0,1, 2, 4,6 and 24 h through the incubation time by making serial dilutions and spreading of 20 μl of each dilution on Mueller-Hinton agar plate (triplicate); colonies range 30-300 CFU/plate was accepted (Miles et al., 1938).

2.3.3. Post antibacterial effect (PAE)

A standard viable counting method was utilized to determine the post antibacterial effect of ceftriaxone against Klebsiella pneumonia (Ozbek Celik et al., 2014).

Ceftriaxone had been dissolved in Mueller-Hinton broth to prepare 10 mg/ml stock solution, then, 10x, 1x and 0.1x MICs concentrations were made and each tube was inoculated 0.1 of bacterial suspension that previously made, then tubes were incubated on 37 ºC for 2 hrs; after incubation, the antibacterial effect of ceftriaxone was removed by

diluting 1 part of cultured Mueller-Hinton broth in 1000 parts of pre-warmed bacteria-free Mueller-Hinton broth then tubes reincubated on 37 ºC for 24 hrs (Aeschlimann and Rybak, 1998).

Bacterial colonies were calculated at 0 hr., 2 hrs. before dilution, 2 hrs. after dilution, 4 hrs. after dilution and 6 hrs. after dilution through the incubation period by making serial dilutions and spreading of 0.1 ml of each dilution on Mueller Hinton agar plate and colonies were calculated as mentioned previously (Miles et al., 1938).

PAE was calculated as (Ozbek Celik et al., 2014):

$$PAE = T - C$$

Where T and C are the time required to increase 1-log10 CFU following 1:1000dilution for the bacteria treated with (T) and without (C) the agents, respectively.

2.3.4. Mutant Prevention concentration (MPC)

An amount of 0.1 ml of freshly prepared 1.5 x 10^8 CFU/ml (equivalent to 0.5 MacFarland) Klebsiella pneumonia suspension was transferred to broth to obtain 10^6 CFU/ml bacterial suspension then incubated on 37 ºC for 2 hrs. to bring Klebsiella pneumonia to the log phase of bacterial growth.

Plates of Mueller-Hinton agar ceftriaxone has been prepared and a calculated aliquot was diluted in previously prepared Mueller-Hinton agar (45-50ºC) to produce different concentrations 4x, 2x, 1x, 0.5x and 0.25x MICs .

Each concentration was poured into a petri dish (triplicate) and 0.1 ml of bacterial suspension was spread on each plate then incubated at 37 ºC for 72 hrs.The lowest antibiotic concentration that recorded no visible growth of bacteria is considered the concentration that prevents bacterial mutations (Blondeau et al., 2009; Aditi Priyadarshini et al., 2019).

Statistics analysis

Data were analyzed statistically using the Microsoft Program (SPSS) .The our finding have been analyzed by using SPSS application to evaluate statistical differences with the Chi-square and the P Values $P \leq 0.05$,the data points had been achieved with Microsoft Excel spreadsheets (Leech et al., 2013).

3. Results

3.1. Isolation and identification

The results of primary isolation and identification of bacteria that caused pneumonia in patient infected with Covid-19 listed in the table (1) showed that the prevalence rate of K. pneumoniae comprises about 32% from all sputum and swap samples against 68% for other unspecific bacterial species.

Table 1: Prevalence percentage of K. pneumonia and other bacteria in lower respiratory tract in patient.

Type of Bacterial isolate	Frequency	Prevalence (%)
Klebsiella pneumoniae	**16**	**32**
Other bacteria	**34**	**68**
Total	**50**	**100**

VITEK® 2 System Identification:

The results of VITEK 2 system identification that all K**.** pneumoniae that had been tested belonged to bacterial gram-negative bacilli card (GNB) to biochemical reactions for 16 Isolates had been achieved an excellent identification level with a probability of 98% based on the manufacturer's technical datasheet and this is consistent with (Nimer et al., 2016).

4.2. Pharmacodynamics analysis :

4.2.1. Minimum Inhibitory Concentration (MIC)

The our results showed that the concentrations 1, 2, 4, 8, 16, 32, 64 , 128 and 256 µg/ml of ceftriaxone are active against 6.25% , 12.5% , 37.5%, 62.5%, 75%, 81.25%, 87.5% and 100% of K. pneumoniae isolates that tested in microdilution assay . The selected isolate has been chosen as a standard isolate to determine further parameters pharmacodynamics of ceftriaxone and for induction of experimental pneumonia where recording 128 µg/ml concentration that inhibited growth of K. pneumonia (256 µg/ml) are killed K. pneumoniae so considers the (MBC) that tested in microdilution assay as shown in the figure (1).

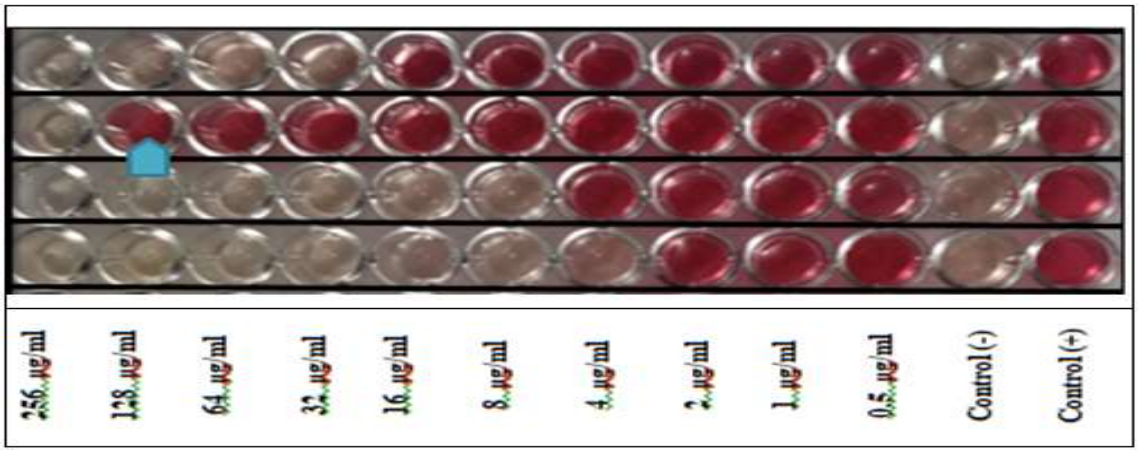

Figure (1): (MIC) and (MBC) of selected isolate is (128 µg/ml) (256 µg/ml) of ceftriaxone respectively against Klebsiella pneumonia Isolates yellow (No growth) pink (growth).

The Minimum Inhibitory Concentration (MIC) plays a key role in the determination of an antibacterial potency (Wiegand et al., 2008). Many methods were used to determine the MIC, but the microdilution assay is the most adopted and accredited method by the European Committee on antimicrobial susceptibility testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) to determine the MIC (Elshikh et al., 2016).

Our results of the MIC revealed that 75% of isolates of K. pneumonia are susceptible and 25% isolates of K. pneumonia are resistance to Ceftriaxone as shown in figure (4-7) whereas the highest MIC value was 128 µg/ml recorded for K. pneumonia and it agreement with (Wang and Xu, 2021).

4.2.2. Bacterial Time kill curve kinetics

Results of time-kill curve kinetics of ceftriaxone are based on the highest MIC recorded from the microdilution MIC assay which was 128 µg/ml respectively for the K**.** pneumoniae strain, the in-vitro concentrations involved in the study were 0.25x MIC, 0.5x MIC, 1x MIC, 2x MICs, 4x MICs.

All concentrations from 4x (MICs) through 2x (MICs) achieved the bactericidal effect by reducing ≥ 3 log10 of the total number of CFU/ml of K**.** pneumoniae as reported at the 24th hour figures, (2).

The area under the time of killing curve of ceftriaxone was calculated and compared to control inoculum growth rate, the difference in the area under the curve values among different treatments were set as an endpoint whereas the lowest area under the curve refers to the highest bactericidal effect as reported in the table (2) .

The results showed that all of 2x (MICs) and 4x (MICs) achieved the highest significant bactericidal effect (P≥ 0.05) in comparison to other treatments, The 1x MIC concentration achieved purely bacteriostatic effect (P≥ 0.05) in comparison to all concentrations and control groups; Both of 0.5x MIC and 0.25x MIC failed to achieve a significant bacteriostatic or bactericidal effect (P≤ 0.05) in comparison to 1xMIC, 2xMIC and 4xMIC.

Our results according to the obtained curves figures, (2) the calculated areas under each one of them tables, (2) both 0.25x MIC and 0.5x MIC concentrations showed no significant antibacterial effect in comparison to the control curve in contrast to 1x MIC that showed a significant bacteriostatic effect against K. pneumonia throughout 24 hrs.; such bacteriostatic effect is expected since the 1x MIC of Ceftriaxone has located within the determined range of K. pneumonia sensitivity toward Ceftriaxone which determined from ≤ 4 to 32µg/ml (Wang et al., 2021) .Time killing curve of each 2x MICs, and 4x MICs concentrations of Ceftriaxone showed a distinguished bactericidal effect at the 24th and 6th hr. of the experiment as same as what reported by (Palmer et al., 1995) whereas they found that Ceftriaxone killed more than 99.9% of K. pneumonia when concentration increase more than two fold in MICs.

Table (2): Area under the time-kill curve of ceftriaxone against K. pneumonia (h*log CFU/ml).

Antibiotics	Control	0.25x MIC	0.5x MIC	1x MIC	2x MIC	4x MIC
Ceftriaxone MIC= 128 µg/ml	**132.302 ± 0.13 A**	**112.47 ± 0.42 A**	**98.675 ± 0.44 A**	**67.265 ± 0.24 B**	**42.407 ± 0.32 C**	**24.88 ± 0.14 C**

- Values represent mean ± S.E
- Different letters denoted a significant difference (p≤0.05) among the groups.

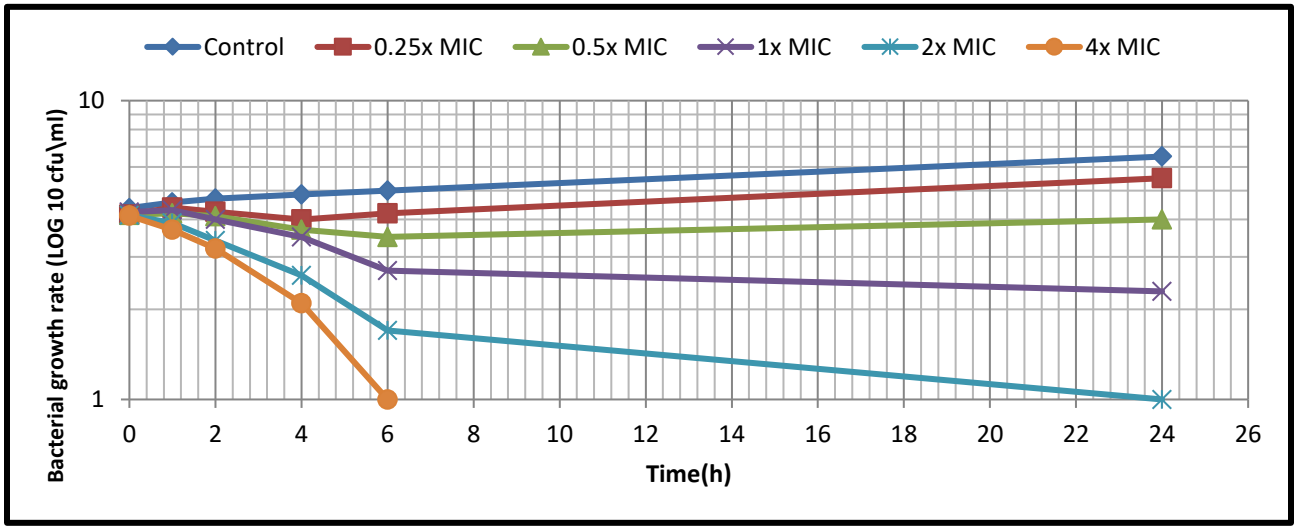

Figure 2: Time kill curve kinetics of Meropenem against K. pneumoniae

4.2.3. Post antibacterial effect (PAE)

The post antibacterial effect of ceftriaxone regarded the difference in the time that consumed by treatment and control culture for 1- log10 increment in colonies count of K. pneumonia, after removal of antibiotics effect by dilution Regarding the MIC value (4 µg/ml), three different concentrations of (0.1x MIC, 1x MIC, and 10x MIC) had been tested against control culture.

Our results also exhibited significant increasing (P≥ 0.05) in the required time for bacterial regrowth by 1Log10 of K. pneumoniae that exposed to 1x MIC and 10x MIC concentrations of antibiotics in comparison to both control and 0.1x MIC cultures .

The time of post-antibiotic effect that recorded in 1x MIC and 10x MIC culture after removal of Ceftrixone effect were 0.51 hr. (30.6 min) and 0.80 hr. (48 min.) respectively as reported in the table (3) .

The factors control the duration of PAE; the type of the antibacterial, the bacterial isolate that is used in, the concentrations of the antibacterial in the medium, and the variety of methodologies of assessment of that effect (Ozbek Celik et al., 2014).

All accomplished times of PAE of Ceftriaxone can be attributed to the non-lethal damage that may be made on K. pneumonia as a result of multiple binding to Penicillin-binding proteins consequently, delaying their catalytic effect (Sauvage and Terrak., 2016).

Our results are completely agreement for Ceftriaxone with many previous study like Craig and Gudmundsson, (1996) that mentioned β-lactam antimicrobials have a short (≥1 h) for aerobic Gram-negative bacilli such as P. aeruginosa, E. coli, and Klebsiella pneumonia.

Table (4-5): Post Antibiotic Effect of Ceftriaxone against K. pneumonia (h.)

Concentration	Control (C)	0.1x MIC	1x MIC	10x MIC
Growth after Ceftriaxone removal (1 log10/hrs.)	4.00 ± 0.01 A	4.10 ± 0.04 A	4.51 ± 0.08 B	4.80 ± 0.09 B
Post Antibiotic Effect (hrs.) (T-C)	N.A	0.10 ± 0.002 A	0.51 ± 0.003 B	0.80 ± 0.0034 B

- Values represent mean ± S.E
- Different letters denoted a significant difference ($p<0.05$) among the groups.
- N.A. (Not Applicable)
- Ceftriaxone (0.1MIC= 6 min , 1 MIC= 30.6 min , 10 MIC= 48 min)

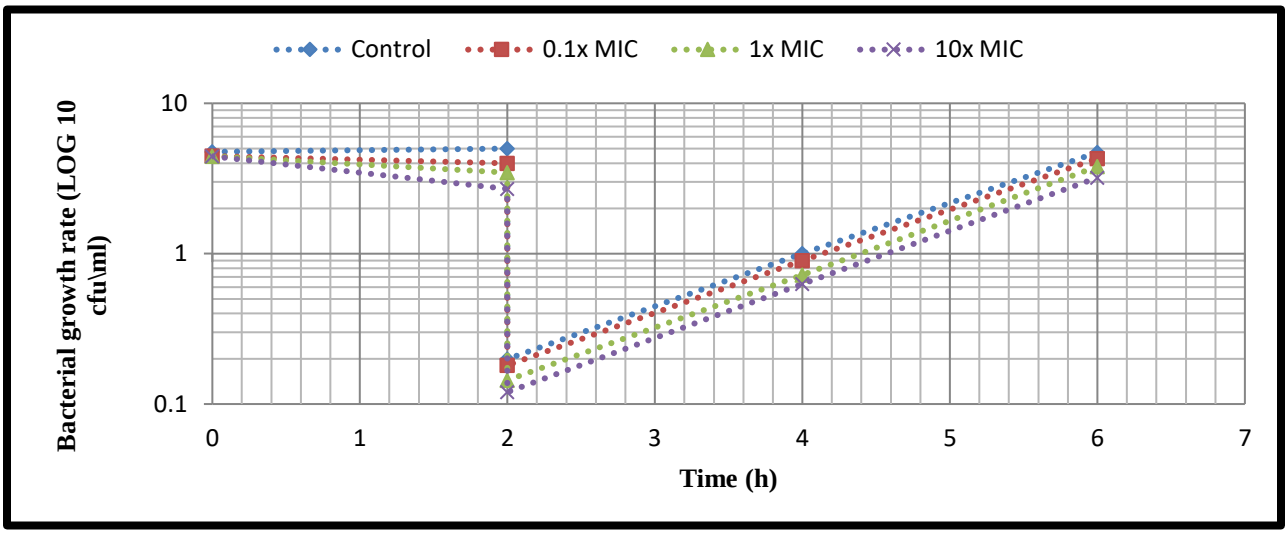

Figure 3: Post antibacterial effect (PAE) curve kinetics of Meropenem against K. pneumonia

4.2.4. Mutant Prevention concentration (MPC)

Our results revealed that there was no visible bacterial growth on the plates that contain 8x MIC, 4x MIC, and 2x MIC of antibiotics in comparison to the control plate. The results of MPC for 1x MIC concentration of antibiotics showed a visible weak growth of K. pneumonia in comparison to the control plate. Also, there was a heavier bacterial growth was appeared in the plate that contains 0.5x MIC concentration of Meropenem in comparison to the plate that contains 1x MIC concentration of antibiotics.

Based on the recorded MIC value that result from our results are (128 µg/ml) of ceftriaxone respectively against K. pneumonia, the obtained observations of our mutant prevention concentration (MPC) after 72 hrs. of incubation were reported in the table (4).

The Mutation prevention index (MPC/MIC) was calculated depending on the recorded results of mutant prevention concentration to the minimum inhibitory concentration of the used isolate of in the test that recoded (2) for ceftiaxone.

Our results reported in the table (4) revealed that the lowest concentration of Ceftriaxone that prevents bacterial mutation were 256 µg/ml (2x MIC); this value is equivalent to the minimum bactericidal concentration obtained from the time killing curve of Ceftriaxone figure (2).

The mechanism that produces (MPC) of most antibiotic groups is not well evaluated as Fluoroquinolones and some other antibiotics (Smith et al., 2003), but, our speculations suppose that the rapid bactericidal mechanism of Ceftriaxone that achieved by binding to Penicillin-binding proteins (PBPs) then after the rapid killing of bacterial population of K. pneumonia due to rapid inhibition of cell wall synthesis; will reduce the chances of bacterial mutation (Zhanel et al., 2007, Sutaria et al., 2018).

Table 4: Mutant preventive concentration of ceftriaxone against Klebsiella pneumonia.

Concentration	Observation of ceftriaxone
Control	+
0.25x MIC	+
0.5x MIC	+
1x MIC	+
2x MIC	-
4x MIC	-
MPC/MIC= 2	

- (+) Visible bacterial growth (-) No bacterial growth

Conclusion:

In this research work, ,we found that ceftriaxone exhibited an efficacious pharmacodynamic profile against K. pneumonia qualifying to be a potential candidate to treat the complicated infections, which K. pneumonia could cause pneumonia and other lower respiratory tract infection. Using ceftriaxone parenteral antibacterial therapy regimens is very efficacious in the treatment of clinical pneumonia.

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