ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Antibacterial Activity Of Cefotaxime Antibiotic.

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ABSTRACT

Cefotaxime is the third generation cephalosporin antibiotic which have efficacy against bacteria. The objective of this research is to observe the antibacterial activity of cefotaxime antibiotic made by Laboratorium of Center for Pharmaceutical and Medicine Technology, at concentration of 10.0; 5.0; 2.5; 1.25; 0.62; 031 and 0.16 ppm against gram negatif bacteria *Escherichia coli* ATCC 25922 and gram positive bacteria *Bacillus subtilis* ATCC6633. Both types of bacteria were prepared in media Luria Bertani Agar to attain old 24 hours and dissolves in 10 ml aquadest sterile. As much as 1 ml of bacteria was mixed into 9 ml of Mueller Hinton Agar media then poured into a sterile petridish. Put paper disc with antibiotic Cefotaxime solution at a concentration above, stored and observed after incubation period of 24 and 48 hours implying a decline in the growth of bacteria. The growth of bacteria *E. Coli* was observed from Cefotaxime concentration of 10 ppm until 1,25 ppm while the growth of bacteria *B. subtilis* was observed from 10.0 ppm until 0.31 ppm. The research conducted showed that Cefotaxime antibiotic is more effective against *B. subtilis* (until effective concentration 0.31 ppm) than against bacteria *E. Coli* (until effective concentration 1. 25 ppm).

Keywords : Cefotaxime, antibacterial, *E. coli, B. subtilis*

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ISSN: 0975-8585

INTRODUCTION

Cefotaxime is the third generation antibiotic of Cephalosporin which have efficacy bactericidal and works by blocking synthesis mukopeptida in the cell wall bacteria. Cefotaxime very stable to hydrolysis beta laktamase, so Cefotaxime used as an alternative first line on bacteria that are resistant to Penicillin. Cefotaxime have a broad spectrum activity against gram positive and gram negative bacteria. Activity Cefotaxime greater against gram negative bacteria while activity against bacteria gram positive smaller, but some Streptococci is highly sensitive to Cefotaxime. Treatment with cefotaxime that is broad spectrum antibiotics can change flora normal of the intestines and causing excessive growth of clostridia . A toxin produced *Clostridium difficile* is a cause of colitis . So need cautious to patients who have indigestion (1).

In laboratory scale, as a broad outline process of making Cefotaxim done with reaction compound 7-ACA (7-aminocephalosporanic acids) and MAEM (2-mercaptobenzothiazolyl) by using a solvent acetone (to dissolve 7-ACA and TEA (tetra etilamin) as a catalyst (2).

An antibiotic is a medicine used to treat, and in some cases can prevent infection by bacteria. Antibiotics can be used for the condition of the disease that relatively mild as acne to potentially life threatening as pneumonia (as one of infections of the fungi). Alexander Flemming in 1927 find the first antibiotic namely Penicillin, and it starts to be used generally 1940, then antibiotic could say that change the world treatment of the disease and reduce the number of pain and death caused by infectious disease dramatically (3).

Resistance antibiotics is the condition when a strain of bacteria in the human body immune to become resistant against antibiotics. Resistance it develops naturally through mutation evolution at random and can also engineered by the use of drugs / antibiotic , chemical or other substances inappropriate, formerly intended to cure or prevent infectious diseases. As a result, the bacteria can survive and reproduce and were hurt. The bacteria can form special security to a certain types of antibiotics, so dangerous those exposed to the disease. Misunderstand often happens in the community is the notion that resistance to drugs certain is a body of people, yet bacteria that is inside the body those who are resistant to treatment, not his body (4).

Microbiology testing conducted by using microorganisms as an indicator testing. In this case microorganism used as the best concentration component of certain on a mixture of chemical complex, to diagnose certain diseaseas, and to test chemicals to determine the potential mutagenic or carcinogenic an ingredient. Uses the antimicrobial (test) antibacterial activity is getting a medical efficient (5).

The antibacterial activity can be done with the diffusion and methods dilution (disc diffusion method) which performed by measuring diameter clear zone that is the way the response inhibition of the growth of bacterium by an antibacterial compound in a sample. The term of the amount of bacteria to test sensibility/sensitivity namely 105-108 CFU/ml (6).

The diffusion is one of the methods often used. The diffusion (test Kirby & Bauer) is where disc paper strain contains a certain number of placed on medium solid has previously inoculated bacteria test on the surface. After incubated the disc zone around be used for measuring the obstacles remedy against the organism (7) should be conducted to determine the activity of antimicrobial agent.

The plate that containing an antimicrobial agent put in a media that have been planted microorganisms that will diffuses in a medium. The clearly indicated arrested growth microorganisms by an agent antimicrobial on the surface media (8). In the test antibacterial activity used gram positive bacteria that is *Bacillus subtilis* ATCC 6633 and bacteria gram negative, namely *Escherichia coli* ATCC 25922, the collection of Center of Pharmacy and Medical Technology - LAPTIAB - BPPT

The purpose of the antibacterial sefotaksim by using the method diffusion in discs paper (disk method) performed to know antibacterial activity cefotaxime produced by the Laboratory of Pharmaceutical and Medical Technology and Center of Biotechnology, Agency for the Assessment and Application of

Technology, Republic of Indonesia against gram positive bacteria *Bacillus subtilis* ATCC 6633 and gram negative bacteria *Escherichia coli* ATCC 25922, and the study was conducted in July - August 2016.

MATERIAL AND INSTRUMENT

Material used

Cefotaxime antibiotic (produced date May 18, 2016), media Luria Bertani Agar, media Mueller Hinton Agar, paper tissue, gram negative bacteria *Escherichia coli*, ATCC 25922, gram Positive bacteria *Bacillus subtilis* ATCC 6633, Ethanol 96%, Ethanol 70%, Aquadest.

Instrument used

Petri dish, test tube 20 ml and rack tube reaction, squash Erlenmeyer 25 ml, pipette 1 ml, fiery Bunsen, needle ose, pinzet. hand sprayer, stationery, matchsticks, paper disc Ø 8 mm, gloves, masks.

EXPERIMENTAL

Testing antibiotic Cefotaxime against gram negative bacteria *Escherichia coli*, ATCC 25922 and gram positive bacteria *Bacillus subtilis* ATCC 6633 done with the method of diffusion. Variation concentration of antibiotics, namely 10,0 ppm, 5.0 ppm; 2.5 ppm; 1.25 ppm; 0,625 ppm; 0,312 ppm and 0.16 ppm. Testing was done with the procedure as follows:

- Prepare the gram negative bacteria culture in media Luria Bertani Agar that for 24 hours, then dissolved in 10 ml sterile aquadest.
- Prepared media Mueller Hinton Agar sterile with size 0,38 grams in 9 ml aquadest. After warm nails added 1 ml solution gram positive bacteria or gram negative bacteria, Shake with the instrument vortex that mixed average, then cast into a sterile petri dish, open a little petri dish cover (± 30%) until media cold and not dewy and immediately cover a petri dish.
- Prepared Cefotaxime antibiotic in solution aquadest by the concentration of the 10,0 ppm, 5.0 ppm, 2.5 ppm, 1.25 ppm, 0.62 ppm, 0.31 ppm and 0.16 ppm
- Put paper disc, which has been given antibiotic Cefotaxime by concentration of the as mentioned above in a petri dish that contains media Mueller Hinton Agar.
- Store in a place protected and viewed the result of their activity against bacteria after incubation for 24 hours and 48 hours, then the activity of Cefotaxime measured and counted.

The antibacterial completed in three times remedial. The results successive shown in Table 1 and Table 2, Figure 1, Figure 2, Figure 3 and Figure 4 as follows :

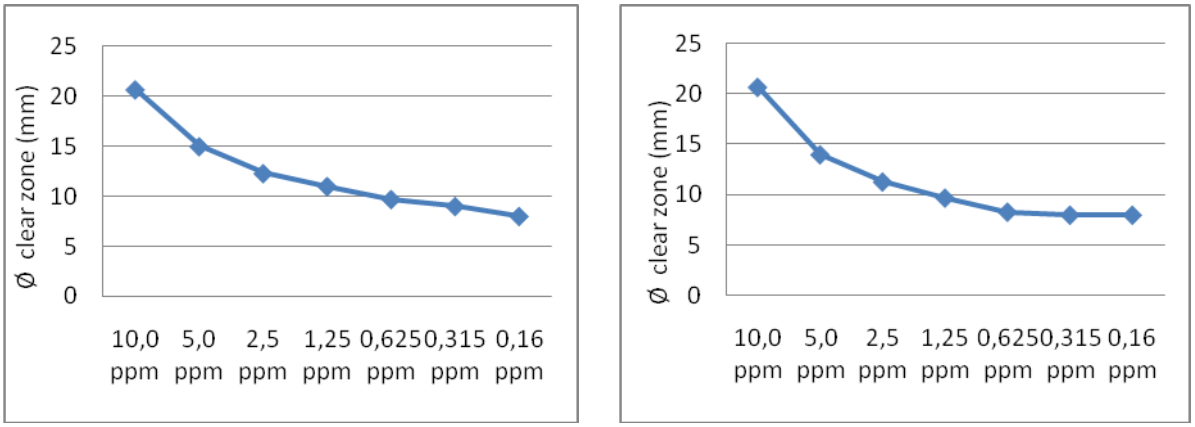
Table 1: Test results antibacteria of Cefotaxime antibiotic on bacteria *Escherichia coli* ATCC 25922 (bacteria gram negative)

Incubation (hours)	Cefotaxime concentratiom (ppm)						
	10.0	**5.0**	**2.5**	**1.25**	**0.62**	**0.31**	**0.16**
24 hours	20,7	15	12,3	11	9,7	9	8
48 hours	20,7	14	11,3	9,7	8,3	8	8

Table 2: Antibacterial test of antibiotc Cefotaxime on bacteria *Bacillus subtilis* ATCC 6633 (bacteria gram positive)

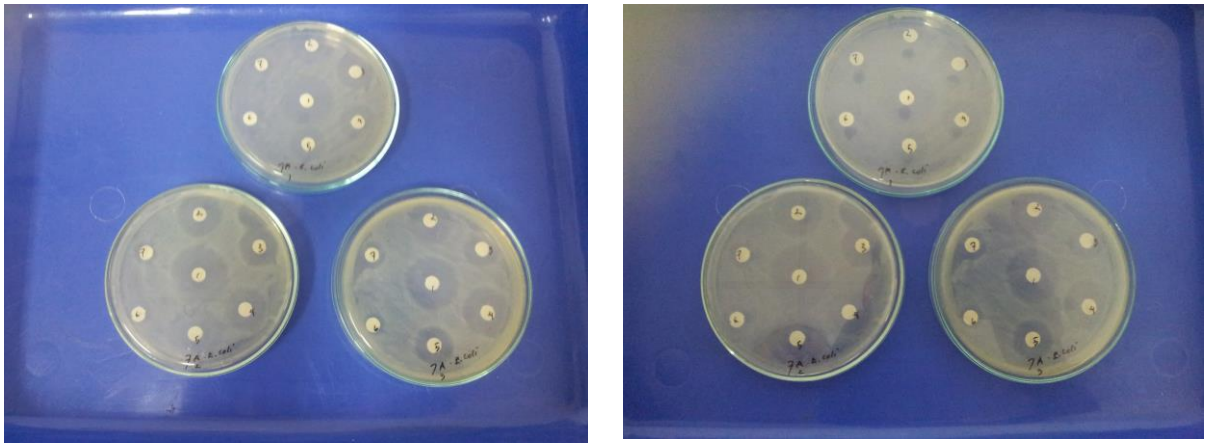
Incubation (hours)	Cefotaxime cnsentration (ppm)						
	10,0	**5,0**	**2,5**	**1,25**	**0,625**	**0,315**	**0,16**
24 hours	32,7	30,7	25,7	21,3	18,6	15,3	11,3
48 hours	27,3	23,7	18	13	8,7	8	8

ISSN: 0975-8585



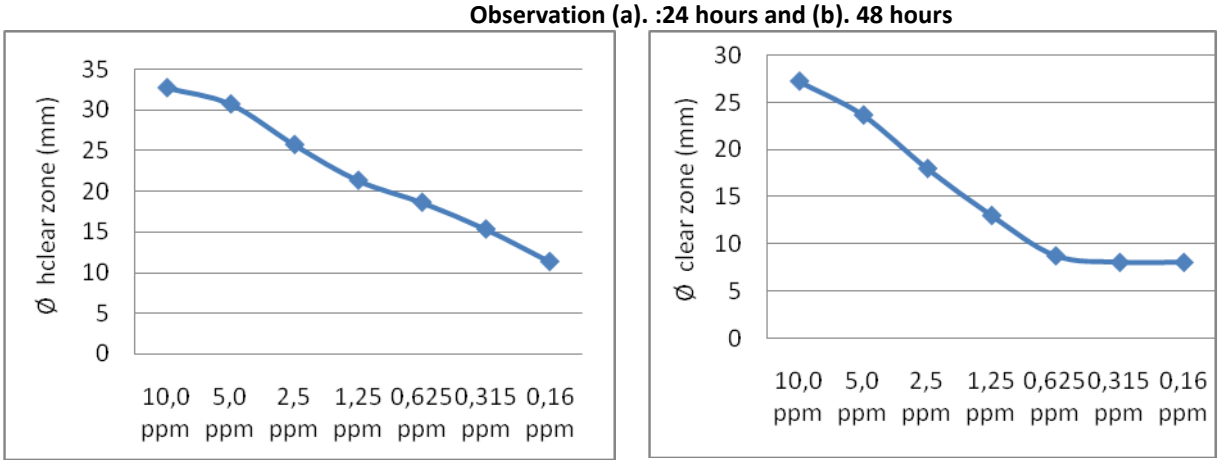
a. b.

Figure 1: The clear zone (mm) Cefotaxime against *Escherichia coli* ATCC 25922 bacteria
Observation (a). :24 hours and (b). 48 hours



a. b.

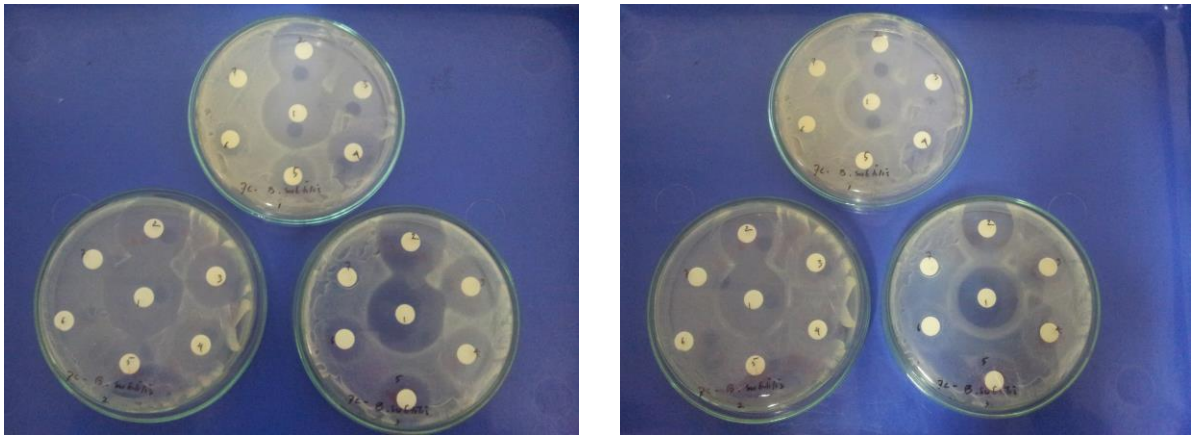
Figure 2: The test antibacterial Cefotaxime with conc. 10,0 ppm – 0,16 ppm against *Escherichia coli* ATCC 25922 bacteria.
Observation (a). :24 hours and (b). 48 hours



a. b

Figure 3: The clear zone (mm) Cefotaxime against *Bacillus subtilis* ATCC 6633 bacteria
Observation (a). :24 hours and (b). 48 hours

ISSN: 0975-8585



a. b.

Figure 4: The test antibacterial Cefotaxime with concetration. 10,0 ppm – 0,16 ppm against *B. subtilis* ATCC 6633 bacteria. Observation (a). :24 hours and (b). 48 hours

RESULT AND DISCUSION

Escherichia coli (*E. coli*) is normal flora in the intestines and can cause illness and is pathogens (9). An infectious disease caused by *E. coli* infection system as urine and diarrhea. Signs and symptom of infection, urinary covering frequency, urinary, disuria, hematuria and Peoria. The results of the experiment obtained is as follows:

The bacterial growth *E. coli* ATCC 25922 in a petri dish hampered by the paper disc containing Cefotaxime antibiotic after the incubation for 24 hours and 48 hours. Power obstruent was not last long, after the incubation for 24 hours paper disc that contains Cefotaxime as many as 0.16 ppm was helpless to prevent the growth of bacteria *E. coli* and when the incubation for 48 hours the growth of bacteria *E. coli* widespread to other places. Concentration Cefotaxime in concentration 0,625 ppm only able to withstand growth *E. coli* small size in diameter (0.3 mm), so it is with the ability.

Cefotaxime to hold back growth *E. coli* in greater concentration Cefotaxime after the incubation for 48 hours investigators that has been carried by Sulastri *et al* (2013). The use of antibiotics for therapy a disease possible cause for *E. coli* still needs to be reconsidered because sensitivity use against Sefotaksim only reached 37. 5 %, so that is still needed further research periodically.

From Figure 1, looks as if both these graphs it looks same, but if be noticed further seen data from Table 1 turn on the incubation 48 hours diameter obstruent (clear zone) Cefotaxime against bacteria *E. coli* does not look back to concentrate Cefotaxime 0,315 ppm, while in the incubation 24 hours zone obstruent was not seen in 0,16 ppm concentration. This reality in line with the previous study of Sulastri *et al* (10) stating that the use of antibiotics for therapeutic disease likely cause *E. coli* needs to be reviewed because sensitivity use against Cefotaxime only 37,5 %, that is still needed further research periodically.

Picture of testing antibacterial Cefotaxime against bacteria *E. coli* seen in Figure 2. The diameter of arrested growth bacteria *E. coli* in testing do not appear to be clear, but apparent that they have had clear zone around paper discs are more narrowed and at the incubation for 72 hours (3 days) clear zone was not seen again.

Bacillus subtilis is gram-positive bacteria, rod-shaped, and naturally often found in soil and vegetation. Some advantage of this bacteria are capable of secrete antibiotics in large numbers out of the cell (11). *B. Subtilis* is bacteria that cause of infection diseases which there are many inside the intestines and it can cause diarrhea that are transmitted through contamination food (Rahmaningsih *et al*., 2012). Antibacterial Cefotaxime test against bacteria *B. subtilis* ATCC 6633 shown in Table 2, Figure 3 and Figure 4 as follows :

From Table 2 it is showed that antibiotic Cefotaxime able to withstand the growth of bacteria *B. subtilis* ATCC 6633 after the incubation for 24 hours and 48 hours, seen by the clear broad zone. Clear zone this after the incubation for 24 hours clearly visible and began disappeared after the incubation for 48 hours is low Cefotaxime concentration (0,625 ppm , 0,315 ppm and 0.16 ppm) and when the incobation 72 hours (3 days) this clear zone piecemeal missing.

B. subtilis have the ability to form a protective endospore giving the bacteria is tolerating an extreme state of *B. subtilis* as obligate anaerobic and not considered as pathogens, although it is contaminants, but rarely cause food poisoning. Application this bacteria in industry quite a lot. *B. subtilis* is one of the most widely used for the production of an enzyme and specialty chemical. According to Rahmaningsih *et al* (12) *B. subtilis* capable to degrades protein in sewage ponds and have a very real different influence. *B subtilis* also can be used as a biodegradation agent because possessing extracellular enzyme to decipher lipid content / fat in liquid waste prawn. *B. subtilis* also able to degrade lipids / fat in a lowly state oxygen (anaerobe facultative).

From Figure 3 it can be seen that Cefotaxime antibiotic able to withstand the growth of bacteria *B. subtilis* ATCC 6633 in a petri dish after the incubation for 24 hours and 48 hours. The clear zone of bacteria is quite wide, although in small concentration (0,625 ppm. 0,315 ppm and 0.16 ppm). After the incubation 48 hours clear zone formed the fading, the diameter of clear zone of small Cefotaxime concentration not seen again (diameter clear zone same in diameter paper disc namely 8 mm).

From Figure 4. the clearly visible clear zone around the paper disc after the incubation 24 hours, especially around the paper disc with Cefotaxime concentration 10,0 ppm (the middle of the picture),Cefotaxime concentration 5,0 ppm (the top of the picture), and other concentration (on the right and left), while after the incubation 48 hours clear zone even in the small Cefotaxime concentration do not appear again.

The testing antibacterial results obtained show that Cefotaxime antibiotic are conducted more effectively inhibit bacteria *B. subtilis* ATCC 6633 growth than bacterial *E. coli* ATCC 25922 growth.

CONCLUSION

- The diameter clear zone of bacteria *Bacillus subtilis* ATCC 6633 wider than diameter clear zone of the bacteria *Escherichia coli* ATCC 25922.
- Diameter clear zone the growth of bacteria *Bacillus subtilis* ATCC 6633 and bacteria *Escherichia coli* ATCC 25922 after the incubation 48 hours apparently increasing compared with the incubation for 24 hours.
- Cefotaxime antibiotic more able to withstand the growth of bacteria *Bacillus subtilis* ATCC 6633 than hold the growth of the bacteria *Escherichia coli* ATCC 25922.

ACKNOWLEGMENT

Word of thanks submitted to the Laboratory of Pharmaceutical and Medical Technology and Center of Biotechnology, Agency for the Assesment and Application of Technology Republic of Indonesia over the license and opportunities given to us to implement testing antibacterial activity of Cefotaxime antibiotic.

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