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EFEK KOMBINASI SEFTRIAKSON DAN KLORAMFENIKOL PADA *Staphylococcus aureus* DARI ISOLAT GANGREN DIABETIK

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ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus*. The combination of Ceftriaxone and Chloramphenicol is often used to eradicate gangrene infection, eventhough, they produce antagonist interaction based on theory. **Objectives:** To evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *Staphylococcus aureus* isolate of Diabetic gangrene. **Material and Methods:** The research was done by using disc diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/ml, 15 µg/ml and 30 µg/ml, respectivelly were tested on *Staphylococcus aureus* culture taken form the diabetic gangrene patients. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed an antibacterial effect lower than single antibiotic. B-lactam antibiotic like Ceftriaxone require that the cell be growing and dividing in order to have a bactericidal action. Chloramphenicol causes a slow growth of *Staphylococcus aureus* and then impairs bactericidal effect of Ceftriaxone. The use of antibiotic combination. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single antibiotic groups on *Staphylococcus aureus* isolate of Gangrene diabetic, therefore it is more rational to use single antibiotic.

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The combination effect of Ceftriaxone and Chloramphenicol on *Staphylococcus aureus* isolate of Diabetic Gangrene

ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus*. The combination of Ceftriaxone and Chloramphenicol is often used to eradicate gangrene infection, eventhough they produce antagonist interaction in theory. **Objectives:** to evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *Staphylococcus aureus* isolate of Diabetic gangrene. **Material and Methods:** The research was done by using disc diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/ml, 15 µg/ml and 30 µg/ml, respectively were tested on *Staphylococcus aureus* culture taken form the diabetic gangrene patients. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed an antibacterial effect lower than single antibiotic. Ceftriaxone require the cell be growing and dividing in order to get bactericidal effect. Chloramphenicol causes a slow growth of *Staphylococcus aureus* and then impairs bactericidal effect of Ceftriaxone. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single antibiotic groups on *Staphylococcus aureus* isolate of Gangrene diabetic.

Keywords: Ceftriaxone; Chloramphenicol; Staphylococcus aureus isolate of Gangrene diabetic.

ABSTRAK

Latar Belakang: Gangrene diabetik merupakan salah satu komplikasi Diabetes melitus yang disebabkan oleh *Staphylococcus aureus*. Kombinasi seftriakson dan kloramfenikol sering digunakan untuk pengobatan infeksi gangrene, meskipun secara teori menghasilkan interaksi antagonis. **Tujuan:** membuktikan potensi seftriakson, kloramfenikol dan kombinasinya pada isolat *Staphylococcus aureus* dari gangrene diabetik. **Bahan dan Metode:** Penelitian dilakukan dengan metode difusi cakram dengan media Muller Hinton. Seftriakson, kloramfenikol dan kombinasinya dengan dosis 7,5 g/ml, 15 g/ml dan 30 g/ml, masing-masing diuji pada kultur *Staphylococcus aureus* dari gangrene diabetes. Efek antibakteri diamati dengan mengukur zona hambat pada kultur bakteri. Hasil penelitian diuji secara statistik dengan One Way ANOVA (p=0,05) dilanjutkan dengan uji Beda Nyata Terkecil (BNT). **Hasil:** Kombinasi seftriakson dan kloramfenikol menunjukkan efek antibakteri lebih rendah dibandingkan antibiotik tunggal. Antibiotik seftriakson memerlukan kondisi sel tumbuh dan membelah agar memiliki aksi bakterisida. Sementara kloramfenikol menghasilkan hambatan pertumbuhan *Staphylococcus aureus* dan mengganggu efek bakterisidal dari seftriakson bila dikombinasi. **Simpulan:** Kombinasi seftriakson dan kloramfenikol memiliki efek antibakteri yang lebih rendah dibandingkan dengan kelompok antibiotik tunggal pada isolat *Staphylococcus aureus* diabetes Gangrene.

Kata kunci: seftriakson; kloramfenikol; *staphylococcus aureus*, gangrene diabetik.

INTRODUCTION

Diabetic foot infection is a major cause of hospitalized patient in developing country (Gadapalli, 2006). In 2009, research at Makasar Hospital Indonesia indicated about 20,61% hospitalized patients were caused Diabetic foot infection (Hasan, 2009). Severe diabetic foot infections and slow handled can lead to gangrene diabetic. Variety of bacteria causing infection of diabetic gangrene is a gram positive bacteria, gram negative and anaerobic bacteria (Brand, 2000). Research in the New England Deaconess Hospital showed gangrene diabetic infection always result more than 2 groups of bacteria (Rowbathan, 1982). *Staphylococcus aureus, Streptococcus sp, Pseudomonas sp* are the major cause of gangrene infection (Leicter, 1988 in Nana Fitria 2008).

One of pharmacological therapy for gangrene diabetic is giving antibiotics. Antibiotics administration should be match with the culture of gangrene bacteria on the infected wound. On the worsening of diabetic foot infections should be given antibiotics while waiting culture results. In cases of diabetic foot infection, combination of Cephalosporin group and Chloramphenicol (CML) is used to cure the infection. Cephalosporin group are often used for such cases is Ceftriaxone (CTX) or Ceftazidim (Adam, 1985).

The combination of antibiotics which include fixed combination has been proven and recognized as a fixed combination of a drug. Several combinations of antibiotics having bactericidal and bacteriostatic effect are not known exactly due to they are influenced by the dose. Theoretically, the combination of antibiotics is confirmed antagonist, however, it is still used because it is considered not clinically meaningful (Suharjono et al, 1994).

Treatment of diabetic foot infection using the combination of bactericidal antibiotic like Cephalosporin and bacteriostatic antibiotic like CML can produce antagonistic interactions, therefore, it reduced the potency of antibiotics (Mycek, 1997). Based on these statements, it is necessary to evaluate the interaction effects of the combinations antibiotics such as Ceftriaxone (CTX) and Chloramphenicol (CML) against *Staphylococcus aureus* (*S.aureus*) isolate gangrene diabetic.

The combination effect of CTX and CML against *S. aureus* isolate gangrene diabetic can be tested *in vitro* using diffusion method. (Jewetz et al, 2005). Diffusion method is widely used to know the antibiotic effects because these methods are simple, cheap, repetition gives good results (Harmita and Radji, 2005).

Based on explanation above, the objective of the study to know the combination effect of CTX and CML against *S. aureus* isolate of gangrene diabetic using *in vitro* method.

MATERIAL AND METHODS

MATERIALS

S. aureus isolate gangrene diabetic, Muller Hinton media, Ceftriaxone (CTX), Chloramphenicol (CML), Paper disk, Mc-Farland turbidity standard.

METHODS

Bacteria media preparation

Muller Hinton media 3.8% was made by adding sterile distilled water and then boiled until complete dissolution. Muller Hinton media was poured into empty petri dish and left until hardened. Muller Hinton media has hardened put in an incubator with a temperature of 37°C for ± 24 hour to see if there is bacterial contaminants in the media that has been made. If a bacterial contaminant does not found then the media is ready to use.

Antibiotic Preparation

Both CTX and CML were each dissolved with sterile distilled water and then they were further dissolved to obtain 30 µg/ml, 15 µg/ml, 7.5 µg/ml. Furthermore, the combination of CTX and CML were made to obtain three composition, the ratio CTX:CML are (C1) 30 : 7.5 µg/mL, (C2) 15 : 15 µg/mL, and (C3) 7.5 : 30 µg/mL. Paper disc inserted to the antibiotic solution for preparation of antibacterial assay.

Antibacterial Assay

S. aureus was obtained from gangrene diabetic patient of dr. Saiful Anwar Hospital Malang. The bacterial suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5×10^7 cells/ml) tubes. Next, it was diluted to obtain a final 5×10^6 cells/ml. Bacteria was sub-cultured on Muller Hinton media for bacterial propagation (Cruickshank, 1979). The broth was inoculated by the 0.2 µg /ml by *S. aureus* and then added paper disk containing CTX, CML and its combination. Furthermore, the plates were incubated at 37 ± 1°C for 24 h and observed for colony growth. Clear zone was measured by a shove showing bacterial growth inhibition.

Statistical analysis

The experimental results were repeated four times and inhibition zone were measured in mm. All the results were tested using One Way ANOVA test followed Least Significant Difference (LSD). For comparing the combination of antibacterial effect were used techniques Independent T-test (p=0,05).

RESULTS AND DISCUSSION

Inhibition zone diameter of CTX, CML and their combination on *S. aureus* growth can be shown in the Table 1, Figure 1 and Figure 2.

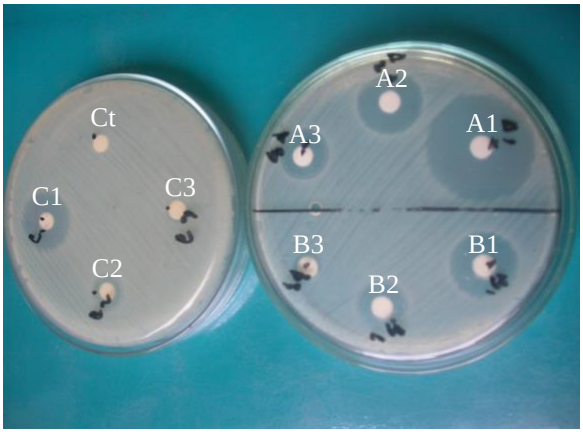

Figure 1. Inhibition zone diameter of Ceftriaxone, Chloramphenicol and their combination on *S. aureus.* (Ct=control, A1=CTX-30, A2=CTX-15, A3=CTX-7.5, B1=CML-7.5, B2=CML-15, B3=30, C1=CTX30-CML7.5, C2= CTX15-CML15, C3= CTX7.5-CML30)

Table 1. Mean inhibition zone diameter of CTX, CML and their combination on *S. aureus* growth

No.	Group	n	Mean ± SD (mm)	☼Antibacterial effect
1	Control (Ct)	4	6.00±0.00	No Effect
	CTX (30 µg/mL/A1)	4	28.96±0.02	Strong
	CML (7,5 µg/mL/B3)	4	7.97±0.24	No Effect
	CTX-CML (30:7.5 µg/mL/C1)	4	17.77±0.32*#$	Moderate
2	Control (Ct)	4	6.00±0.00	No Effect
	CTX (15 µg/mL) (A2)	4	21.00±0.05	Strong
	CML (15 µg/mL) (B2)	4	12.37±0.49	Weak
	CTX-CML (15:15 µg/mL) (C2)	4	14.03±0.22*#$	Weak
3	Control (Ct)	4	6.00±0.00	No Effect
	CTX (7,5 µg/mL) (A3)	4	11.88±0.00	Weak
	CML (30 µg/mL) (B1)	4	17.98±0.05	Moderate
	CTX-CML (7.5:30 µg/mL) (C3)	4	8.45±0.44*#$	No Effect

Note :
*: $p \leq 0.05$ different significantlly (LSD test post hoc ANOVA) compared to control group.
#: $p \leq 0.05$ different significantlly (LSD test post hoc ANOVA) compared to CTX
$: $p \leq 0.05$ different significantlly (LSD test post hoc ANOVA) compared to CML
☼: Antibacterial effect based on Greenwood Classification (1995)

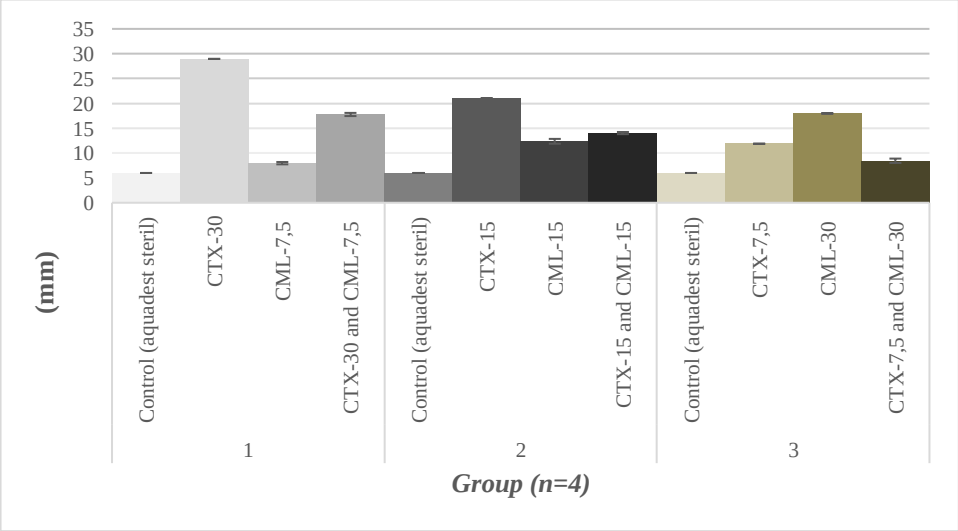

Figure 2. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* growth

Based on these result, a significant decrease of inhibition zone diameter on combination CTX-CML (30:7.5 µg/mL) compare to CTX 30 µg/mL. However, inhibition zone diameter of the combination was higher than CML 7.5 µg/mL. CML showed a low antibacterial effect due to the dose used and the less sensitivity of them to *S. aureus*. The combination of CTX 30 µg/mL and CML 7.5 µg/mL results antagonist interaction. CTX has bactericidal effect whereas CML has bacteriostatic effect. When two antibiotics above are combined, the activity of bactericidal antibiotic will be inhibited by bacteriostatic antibiotic. CTX works through inhibiting the formation of cell wall, meanwhile CML blocking protein synthesis especially at ribosome sub unit 50S (Jewetz et al, 2005).

The second combination CTX-CML (15:15 µg/mL) indicated a significant reduce of inhibition zone diameter compare to CTX 15 µg/mL. On the other hand, inhibition zone diameter of the combination was higher than single CML 15 µg/mL. The combination produces antibacterial effect lower than CTX 15 µg/mL due to antagonist interaction between bactericidal antibiotic and bacteriostatic therefore it reduces their potency as antibiotic, the explanation is same with the previous combination. This combination has lower antibacterial effect than the first combination since the change of their combination dose both in CTX and CML.

For third combination CTX-CML (7.5:30 µg/mL) showed a significant decrease of inhibition zone diameter compare to both of single CTX 7.5 µg/mL and CML 30 µg/mL. This combination has lower antibacterial effect than two combination previous and according to Greenwood it does not have antibacterial effect. The interaction was antagonist same with two combination noted previously, it was caused by the dose of this combination. CTX was more susceptible to *S. aureus* in fact the dose decreased by 7.5 µg/mL whereas CML was less sensitive to gram positive bacteria the dose was increased up to 30 µg/ml. It results lower antibacterial effect than single antibiotic.

Table 2. Mean inhibition zone diameter of combination CTX and CML in multiple composition on *S. aureus* growth

Combination	Group	n	Mean ± SD (mm)	☼Antibacterial effect
C1	CTX-CML (30:7,5 µg/mL) (C1)	4	17.77±0.32*#	Moderate
C2	CTX-CML (15:15 µg/mL) (C2)	4	14.03±0.22$#	Weak
C3	CTX-CML (7.5:30 µg/mL) (C3)	4	8.45±0.44$*	No Effect

Note :
$: $p \leq 0.05$ different significantlly compared to combination 1
*: $p \leq 0.05$ different significantlly compared to combination 2
#: $p \leq 0.05$ different significantlly compared to combination 3
☼: Antibacterial effect based on Greenwood Classification Tabel (1995)

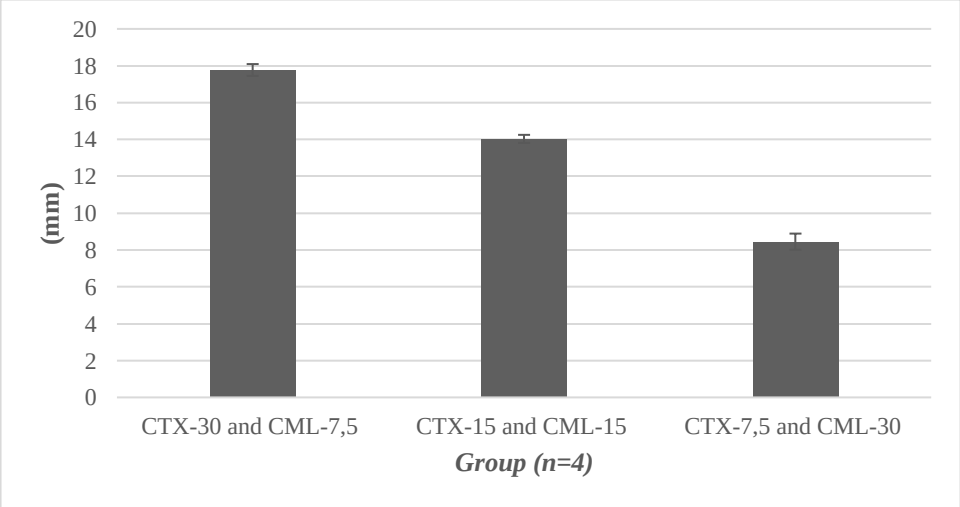

Figure 3. Mean inhibition zone diameter of combination CTX and CML in multiple dose on *S. aureus* growth

Based on the results of this experiment, it was obtained that the first combination has stronger antibacterial effect than two combination others. It is caused by the difference of composition bactericidal and bacteriostatic antibiotic used for each combination. The combination with high dose of CTX and low dose of CML results a strong antibacterial effect on *S. aureus*. The antagonist interaction occurs but according to Greenwood it has moderate antibacterial effect. The higher doses of CTX in the combination result the higher antibacterial effect on *S. aureus*.

Combination of CTX-CML indicated an antibacterial effect lower than single antibiotic in many composition. CTX is bactericidal and CML is bacteriostatic, when they are combined result a decrease of antibacterial potency. ß-lactam antibiotic like CTX require that the cell be growing and dividing in order to have a bactericidal action. CML cause slow growth of *S. aureus* and then impairs bactericidal effect of CTX (Chambers, 2003).

Growth conditions also will affect activity of bactericidal agents. Many antibiotics, and especially ß-lactams, require that cells be growing and dividing in order to have a bactericidal action. Slow growth impairs bactericidal activity. Conditions in the host at the site of infection that favor no growth or slow growth of bacteria will disrupt an antibiotic's bactericidal action. Thus, whether a bactericidal or a bacteriostatic effect is achieved can vary depending on not only the antibiotic but also the organisms and conditions of growth (Chambers, 2003).

Antibiotic was classified as bacteriostatic or bactericidal (Setiabudi, 2007). Bacteriostatic inhibits replication and growth of bacteria, therefore prevent spread of infection. Meanwhile immun system attack, mobilize and eliminate pathogen bacteria. If drug is released before the immun system catch microorganisms, they can live and start the second cycle of infections (Mycek, 1997). The experimental laboratory showed that growth of bacteria was inhibited by administration of bacteriostatic antibiotic. Microorganisms can live although there were exposed by bacteriostatic drugs. On the other hand, giving of bactericidal drugs can kill bacteria and shows a decrease of bacteria number lived.

Mixed infection caused by several groups of bacteria like in gangrene diabetic infection results the use of combination antibiotic (Adam, 1985). Antibiotic combination of CTX and CML is used to increase effectivity on gangrene diabetic infection. The combination was selected to wide spectrum of bacteria also. This condition produces a drug interactions was antagonistic effect. Pharmacologically, this case is classified in pharmacodynamic interaction, furthermore, it can influence the activity of drugs on microrganisms. The synergistic interaction results an increase of drug potency but antagonistic interaction produces a decrease of drug effect (Joenoes, 1994).

Antibiotics are effective drug in infection diseases therapy due to their potency to kill microorganisms. In many cases, antibiotic treatment requires controlling of dose to eradicate microorganisms so the body can tolerate it (Jewetz et al, 2005).

Selection of antibiotic for infection therapy requires the knowledge about microorganisms identity and their sensitivity to antibiotic, place of infection, antibiotic safety, patient factor and cost (Mycek, 1997). However, some seriously ill patient require empiric therapy that is giving antibiotic as soon as possible which can fight microorganisms both positive and negative gram while waiting the culture results (Brand, 2000).

Eventhough this is practical, the classification too simple because antibiotic are bacteriostatic for one organisms and are bactericidal to other organisms. For example, CMX is bacteriostatic to gram-negative rods but it is bactericidal to *Pneumococus* sp (Mycek, 1997).

In addition, bactericidal activity is not an invariable property of an antibiotic, it can depend upon the organism and the growth conditions. For example, *S. aureus* is not killed by protein synthesis inhibitors like CML and Erythromycin (ERT), which are classical bacteriostatic agents and which target the ribosome. *Streptococcus pneumoniae*, on the susceptible organism. It is readily killed by these and other antibiotics that are bacteriostatic against hardier bacterial species. Like most bacteria, *S. aureus* and *S. pneumoniae* are killed by cell wall inhibitors, Penicillin and Vancomycin, as well as by the Fluoroquinolones, which are classical bactericidal agents. These agents *in vitro* do not produce 99.9% kill of enterococci within the 24-hour time frame, therefore they are considered to be only bacteriostatic against this organism (Chambers, 2003). The continue between bactericidal activity and bacteriostatic activity that varies depending on the antibiotic, the bacterial species or isolate, and growth conditions (Chambers, 2003).

CONCLUSION

Combination of CTX and CML has antibacterial effect lower than the single antibiotic groups on *S. aureus* isolate Gangrene diabetic. The combination of high dose of CTX and low dose of CML shows antibacterial effect in the moderate category on *S. aureus* isolate diabetic gangrene.

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CONFLICT OF INTEREST

The author declared no conlict of interest

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ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus*. The combination of Ceftriaxone and Chloramphenicol is often used to cure gangrene infection, even though, they produce antagonist interaction based on theory. **Objectives:** To evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *Staphylococcus aureus* isolate of Diabetic gangrene. **Material and Methods:** The research was done by using disc diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/ml, 15 µg/ml and 30 µg/ml, respectively were tested on *Staphylococcus aureus* culture taken form the diabetic gangrene patients. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed an antibacterial effect lower than Ceftriaxone. ß-lactam antibiotic like Ceftriaxone require that the cell be growing and dividing in order to have a bactericidal action. Meanwhile, Chloramphenicol causes a slow growth of *Staphylococcus aureus* and impairs bactericidal effect of Ceftriaxone if they are combined. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single antibiotic groups on *Staphylococcus aureus* isolate of Gangrene diabetic and the type of interaction is antagonistic.

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ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus (S.aureus)*. The combination of Ceftriaxone and Chloramphenicol is used to cure infection, however, their interaction has not been known exactly. **Objectives:** to evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *S. aureus* isolate of Diabetic gangrene. **Material and Methods:** The study was performed by using diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/mL, 15 µg/mL and 30 µg/mL, respectively were tested on *S. aureus* isolate of Diabetic gangrene. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. The interaction of antibacterial was analyzed using AZDAST method. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed antibacterial activity lower than single antibiotic. Antagonistic interaction is resulted by their combination. Ceftriaxone require the cell be growing and dividing in order to get bactericidal effect. Chloramphenicol inhibit growth of *S. aureus* and impairs bactericidal effect of Ceftriaxone. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single Ceftriaxone on *S. aureus* isolate of Gangrene diabetic. The type of interaction antibiotic is antagonistic

Keywords: Ceftriaxone; Chloramphenicol; Staphylococcus aureus isolate of Gangrene diabetic.

ABSTRAK

Latar Belakang: Gangrene diabetik merupakan salah satu komplikasi Diabetes melitus yang disebabkan oleh *S. aureus*. Kombinasi seftriakson dan kloramfenikol sering digunakan untuk pengobatan infeksi gangrene, meskipun interaksinya belum diketahui secara pasti. **Tujuan:** membuktikan potensi seftriakson, kloramfenikol dan kombinasinya pada isolat *S. aureus* dari gangrene diabetik. **Bahan dan Metode:** Penelitian dilakukan dengan metode difusi cakram dengan media Muller Hinton. Seftriakson, kloramfenikol dan kombinasinya dengan dosis 7,5 g/mL, 15 g/mL dan 30 g/mL, masing-masing diuji pada kultur *S. aureus* isolat gangrene diabetes. Efek antibakteri diamati dengan mengukur zona hambat pada kultur bakteri. Tipe interaksi dianalisa dengan metode AZDAST. Hasil penelitian diuji secara statistik dengan One Way ANOVA (p=0,05) dilanjutkan dengan uji Beda Nyata Terkecil (BNT). **Hasil:** Kombinasi seftriakson dan kloramfenikol menunjukkan efek antibakteri lebih rendah dibandingkan antibiotik tunggal seftriakson. Interaksi antagonis dihasilkan dari kombinasi antibiotic ini. Seftriakson memerlukan kondisi sel tumbuh dan membelah agar sebagai agen bakterisida. Sementara kloramfenikol menghambat pertumbuhan *S.aureus* dan mengganggu efek bakterisidal dari seftriakson bila dikombinasi. **Simpulan:** Kombinasi seftriakson dan kloramfenikol memiliki efek antibakteri yang lebih rendah dibandingkan dengan antibiotik seftriakson tunggal pada isolat *S. aureus* diabetes Gangrene. Tipe interaksi antibiotic bersifat antagonis.

Kata kunci: seftriakson; kloramfenikol; *staphylococcus aureus*, gangrene diabetik.

INTRODUCTION

Diabetic foot infection is a major cause of hospitalized patient in developing country (Złoch et al., 2021). In 2009, research at Makassar Hospital Indonesia indicated about 20,61% hospitalized patients were caused by diabetic foot infection (Rohmah, 2019). Severe diabetic foot infections and late handled can lead to gangrene diabetic (Aulia et al., 2019). Variety of bacteria causing infection of diabetic gangrene is a gram positive, gram negative and anaerobic bacteria (Prasetya et al., 2019). Research in the New England Deaconess Hospital indicated gangrene diabetic infection always caused more than 2 groups

of bacteria (Caroline, 2016). *Staphylococcus aureus*, *Streptococcus sp*, *Pseudomonas sp* are the major cause of gangrene infection (Yutaka, 2020).

One of pharmacological therapy for gangrene diabetic is antibiotics. The administration of antibiotics should be appropriate with the culture of gangrene bacteria on the infected wound. On the worsening of diabetic foot infections should be given antibiotics while waiting culture results. In cases of diabetic foot infection, combination of antibiotic is used to cure the infection (Caroline, 2016; Otoupal et al., 2021) Cephalosporin group are often used for such cases is Ceftriaxone (CTX) or Ceftazidim (Caroline, 2016; Jahani et al., 2017). The combination of antibiotics which include fixed combination has been proven and recognized as a fixed drug combination. Several combinations of antibiotics having bactericidal and bacteriostatic effect are not known exactly due to they are influenced by the dose (Navarro-Pérez et al., 2021). Theoretically, the combination of antibiotics is confirmed antagonist, however, it is still used because it is considered not clinically meaningful (Ocampo et al., 2014; Otoupal et al., 2021).

Treatment of diabetic foot infection using the combination of bactericidal antibiotic like Ceftriaxone (CTX) and bacteriostatic antibiotic like Chloramphenicol (CML) can produce antagonistic interactions based on theory (Navarro-Pérez et al., 2021; Złoch et al., 2021). Their interaction will reduce the efficacy of antibiotic. The study can be performed using diffusion method and type interaction with Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) (Ziaei-Darounkalaei et al., 2016). Based on explanation above, the objective of the study was to evaluate the combination effect of CTX and CML against *S. aureus* isolate of gangrene diabetic including the type of interaction.

MATERIAL AND METHODS

MATERIALS

S. aureus isolate gangrene diabetic (Dr. Saiful Anwar Hospital, Malang), Muller Hinton media (Sigma-Aldrich), CTX (Orchid Pharma), CML (Kimia Farma), Paper disk (Merck), Mc-Farland turbidity standard (Sigma-Aldrich).

METHODS

Bacteria media preparation

Muller Hinton media 3.8% was made by adding sterile distilled water and then boiled until complete dissolution. Muller Hinton media was poured into empty petri dish and left until hardened. Muller Hinton media has hardened put in an incubator with a temperature of 37°C for ± 24 hour to evaluate bacterial contaminants in the media that has been made. If a bacterial contaminant does not found therefore the media is ready to use.

Antibiotic Preparation

Both CTX and CML were each dissolved with sterile distilled water and then they were further dissolved to obtain 30 µg/ml, 15 µg/ml, 7.5 µg/ml. Furthermore, the combination of CTX and CML were made

to obtain three composition, the ratio CTX:CML are (C1) 30 : 7.5 µg/mL, (C2) 15 : 15 µg/mL, and (C3) 7.5 : 30 µg/mL. Paper disc was inserted to the antibiotic solution for preparation of antibacterial assay.

Antibacterial Assay (Referensi???)

S.aureus suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5 x 10^7 cells/ml) tubes. Next, it was diluted to obtain a final 5 x 10^6 cells/ml. Bacteria was sub-cultured on Muller Hinton media for bacterial propagation (Solomon and Isaac, 2018). The broth was inoculated by the 0.2 µg /ml by *S. aureus* and then added paper disk containing CTX, CML and its combination. Furthermore, the plates were incubated at 37 ± 1°C for 24 h and observed for colony growth. Clear zone was measured by a caliper showing zone of inhibition (ZOI). Antibacterial activity is classified based on Greenwood, 1995 in Ramadheni et al., 2018: none (less than 10 mm), weak (11-15 mm), moderate (16-20 mm), potent (more than 20 mm). Meanwhile, percent of inhibition was calculated based on formula

$$\text{Percent of inhibition} = \frac{\text{ZOI sample} - \text{ZOI control}}{1.5 \text{ ZOI potent (30 mm)}} \times 100\ \%$$

The IC_{50} value was calculated by linear regression curve fit using SPPS version 16.0

Analysis of antibacterial interaction

Interaction were analyzed using Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) method (Ziaei-Darounkalaei et al, 2016). Type of interaction are synergistic (AB >A&B and </> AA and/or BB), potentiation (one of A/B=0 and AB>A&B and >/< AA and/or BB), antagonistic (AB < A or B), additive (AB=AA and/or BB) and not distinguishable (AB = A or B)

Statistical analysis

The experimental results were replicated four times and the data are expressed as the mean ± SD. The data were tested using One Way ANOVA test and followed by Least Significant Difference (LSD, p=0,05) test.

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial activity of compound was showed by inhibition of bacterial growth. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* isolate of gangrene diabetic can be seen in the Table 1-2, and Figure 1-2.

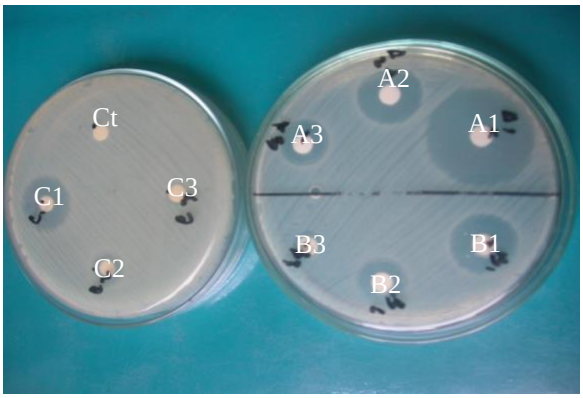

Figure 1. Inhibition zone diameter of Ceftriaxone, Chloramphenicol and their combination on *S. aureus*. isolate of gangrene diabetic (Ct=control, A1=CTX-30, A2=CTX-15, A3=CTX-7.5, B1=CML-7.5, B2=CML-15, B3=30, C1=CTX30-CML7.5, C2= CTX15-CML15, C3= CTX7.5-CML30)

Table 1. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* isolate of gangrene diabetic

No.	Group	n	Mean ± SD (mm)	Antibacterial effect*
1	Control (Ct)	4	6.00±0.00[a]	None
	CTX-30 (A1)	4	28.96±0.02[b]	Potent
	CML-7,5 (B3)	4	7.97±0.24[c]	None
	CTX-30:CML-7.5 (C1)	4	17.77±0.32[d]	Moderate
2	Control (Ct)	4	6.00±0.00[a]	None
	CTX-15 (A2)	4	21.00±0.05[b]	Potent
	CML-15 (B2)	4	12.37±0.49[c]	Weak
	CTX-15:CML-15 (C2)	4	14.03±0.22[c]	Weak
3	Control (Ct)	4	6.00±0.00[a]	None
	CTX-7,5 (A3)	4	11.88±0.00[b]	Weak
	CML-30 (B1)	4	17.98±0.05[c]	Moderate
	CTX-7,5:CML-30 (C3)	4	8.45±0.44[d]	None

Note :
a,b,c : different letter show the different effect (LSD, $p<0.05$ %)
* : antibacterial effect based on Greenwood classification, 1995 in Ramadheni et al (2018)

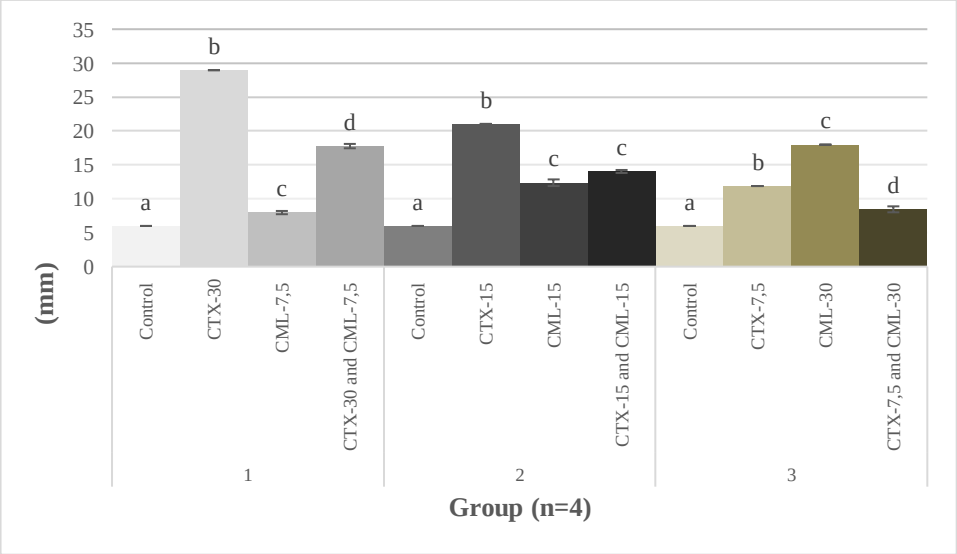

Figure 2. Inhibition zone diameter of CTX, CML and their combination on *S. aureus*

Table 2. Inhibitory concentration-50 (IC_{50}) of antibiotic on *S.aureus* isolate of gangrene diabetic

Antibiotic	Concentration (μg/mL)	n	Mean ± SD (mm)	% Inhibition	IC_{50} (μg/mL)
CTX	0	4	6.00±0.00[a]	0.00	
	7.5	4	11.88±0.00[b]	19.60	
	15	4	21.00±0.05[c]	50.00	18.32
	30	4	28.96±0.02[d]	76.53	
CML	0	4	6.00±0.00[a]	0.00	
	7.5	4	7.97±0.24[a]	6.56	37.17
	15	4	12.37±0.49[b]	21.23	
	30	4	17.98±0.05[c]	39.93	

Note :
a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

Based on these result, the first composition indicated a significant decrease of inhibition zone diameter on combination CTX-CML (30:7.5 μg/mL) compare to CTX 30 μg/mL. However, inhibition zone diameter of the combination was higher than CML 7.5 μg/mL and it has a moderate activity. CML showed a low antibacterial effect due to the dose used and the less sensitivity of them to *S. aureus* (Yutaka, 2020; Alsherbiny et al., 2021).

The second combination CTX-CML (15:15 μg/mL) indicated a significant reduce of inhibition zone diameter compare to CTX 15 μg/mL. On the other hand, inhibition zone diameter of the combination was higher than single CML 15 μg/mL and it has a weak activity. CTX more potent than CML at the same dose, it supported also with the percent inhibition and IC_{50} value which is showed at table 2. CTX has IC_{50} value lower than CML, it means CTX more potent to inhibit *S.aureus* growth compare to CML. For third combination CTX-CML (7.5:30 μg/mL) showed a significant decrease of inhibition zone diameter compare to both of single CTX 7.5 μg/mL and CML 30 μg/mL. This combination has lower antibacterial activity than two combination mentioned previous and according to Ramadheni et al., 2018, it does not have antibacterial effect. Three combination of CTX and CML are disadvantage since the potency of antibacterial decreased until it has no activity. The first combination has antibacterial effect stronger than two combination others. It is caused by the difference of composition bactericidal and bacteriostatic which is used for each combination. CTX has bactericidal effect whereas CML has bacteriostatic effect. When two antibiotics above are combined, the activity of bactericidal antibiotic will be inhibited by bacteriostatic antibiotic meaningful (Ocampo et al., 2014; Navarro-Pérez et al., 2021). CTX works through inhibiting the formation of cell wall, meanwhile CML blocking protein synthesis especially at ribosome sub unit 50S (Eyler and Shvets, 2019; Inayah et al., 2020). The combination with high dose of CTX and low dose of CML results a strong antibacterial effect on *S. aureus.*

Mixed infection caused by several groups of bacteria like in gangrene diabetic infection results the use of combination antibiotic (Caroline, 2016; Złoch et al., 2021). Antibiotic combination of CTX and CML

is used to increase efficacy on gangrene diabetic infection. The combination was selected to expand the spectrum of bacteria also (Kothari et al., 2022). Actually, the aims of antibacterial combination to increase potency, extend spectrum, reduce resistance and toxicity (Alsherbiny et al., 2021; Kothari et al., 2022). However, incorrect selection of antibacterial combination and the dose produce therapy failure (Gilbert et al., 2010; Parkunan et al., 2019).

Interaction of antibacterial CTX and CML

The interaction of CTX and CML is analyzed using AZDAST methods, the result can be seen at table 3

Table 3. Interaction of CTX and CML combination on *S. aureus*

Antibiotic	n	Mean ± SD (mm)	Antibacterial effect*	Type of interaction#
CTX-7.5	4	11.88±0.00[a]	Weak	
CML-7.5	4	7.97±0.24[b]	None	Antagonistic
CTX-7.5 + CML-7.5	4	9.02±0.13[b]	None	
CTX-7.5 + CTX-7.5	4	21.00±0.05[c]	Potent	
CML-7.5 + CML-7.5	4	12.37±0.49[a]	Weak	
CTX-15	4	21.00±0.05[a]	Potent	
CML-15	4	12.37±0.49[b]	Weak	Antagonistic
CTX-15 + CML-15	4	14.03±0.22[b]	Weak	
CTX-15 + CTX-15	4	28.96±0.02[c]	Potent	
CML-15 + CML-15	4	17.98±0.05[d]	Moderate	

Noted

a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

* : antibacterial effect based on Greenwood classification, 1995 in Ramadheni et al (2018)

: type of interaction based on AZDAST method

The combination produces antibacterial effect lower than single antibiotic due to antagonistic interaction between bactericidal antibiotic and bacteriostatic, therefore it reduces their potency as antibiotic. ß-lactam antibiotic like CTX require the cell be growing and dividing in order to have a bactericidal action. CML cause slow growth of *S. aureus* furthermore it impairs bactericidal effect of CTX (Ocampo et al., 2014; Navarro-Pérez et al., 2021). The combination CTX and CML result antagonistic interaction based on AZDAST method.

Conditions of bacterial growth affect the activity of bactericidal agents also. Conditions at the site of infection that favor no growth or slow growth of bacteria will disrupt bactericidal action. Thus, whether a bactericidal or a bacteriostatic effect is achieved can vary depending on not only the antibiotic, however, the organisms and the growth conditions also (Eyler and Shvets, 2019; Navarro-Pérez et al., 2021). Antibiotic was classified as bacteriostatic and bactericidal sketchily, bacteriostatic inhibits replication and growth of bacteria. Meanwhile bactericidal kill bacteria and reduce of bacteria number lived (Sari et al., 2018). Even though the classification is simple, in some case antibiotic is bacteriostatic for one organisms and are bactericidal to other organisms. For example, CML is bacteriostatic to gram-negative rods but it is bactericidal to *Pneumococus* sp (Sood, 2016; Lorenzo, 2019).

The experimental laboratory indicated the growth of bacteria was inhibited by bacteriostatic. Microorganisms can live although there were exposed by bacteriostatic drugs. On the other hand, giving of bactericidal drugs can kill bacteria and decrease of bacteria number lived (Otoupal et al., 2021). Mixed infection caused by several group bacterial such as gangrene diabetic induces the use of antibiotic combination (Caroline, 2016).

Antibiotics are effective drug in the therapy of infection diseases due to their potency to kill microorganisms. In many cases, antibiotic treatment requires controlling of dose to eradicate microorganisms furthermore the body can tolerate it (Paterson et al., 2016).

Selection of antibiotic for infection therapy requires the knowledge about microorganism identity and their sensitivity to antibiotic, place of infection, antibiotic safety, patient factor and cost (Gilbert et al., 2010). However, some seriously ill patient require empiric therapy that is giving antibiotic as soon as possible while waiting the culture results (Prasetya et al., 2019).

In addition, bactericidal activity is not an invariable property of an antibiotic, it can depend upon the organism and the growth conditions. For example, *S. aureus* is not killed by protein synthesis inhibitors like CML and Erythromycin (ERT), which are classical bacteriostatic agents having target at the ribosome (Eyler and Shvets, 2019; Schulte-Werning et al., 2021). The most of bacteria, *S. aureus* and *S. pneumoniae* are killed by cell wall inhibitors such as Penicillin and Vancomycin, as well as by the Fluoroquinolones, which are classical bactericidal agents (Navarro-Pérez et al., 2021). These agents *in vitro* do not produce 99.9% kill of Enterococci within the 24-hour time frame, therefore they are considered to be only bacteriostatic against this organism (Gilbert et al., 2010; Ocampo et al., 2014). The continue between bactericidal activity and bacteriostatic that varies depending on the antibiotic, the bacterial species or isolate, and growth conditions (Ocampo et al., 2014; Paolo et al., 2014).

CONCLUSION

Combination of CTX and CML has antibacterial effect lower than the single antibiotic groups on *S. aureus* isolate Gangrene diabetic and the type of interaction is antagonistic. The combination of high dose of CTX and low dose of CML shows antibacterial effect in the moderate category.

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No declared.

CONFLICT OF INTEREST

The author declared no conlict of interest

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EFEK KOMBINASI SEFTRIAKSON DAN KLORAMFENIKOL PADA *Staphylococcus aureus* DARI ISOLAT GANGREN DIABETIK

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ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus*. The combination of Ceftriaxone and Chloramphenicol is often used to cure gangrene infection, even though, they produce antagonist interaction based on theory. **Objectives:** To evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *Staphylococcus aureus* isolate of Diabetic gangrene. **Material and Methods:** The research was done by using disc diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/ml, 15 µg/ml and 30 µg/ml, respectively were tested on *Staphylococcus aureus* culture taken form the diabetic gangrene patients. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed an antibacterial effect lower than Ceftriaxone. ß-lactam antibiotic like Ceftriaxone require that the cell be growing and dividing in order to have a bactericidal action. Meanwhile, Chloramphenicol causes a slow growth of *Staphylococcus aureus* and impairs bactericidal effect of Ceftriaxone if they are combined. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single antibiotic groups on *Staphylococcus aureus* isolate of Gangrene diabetic and the type of interaction is antagonistic.

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The combination effect of Ceftriaxone and Chloramphenicol on *Staphylococcus aureus* isolate of Diabetic Gangrene

ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus (S.aureus)*. The combination of Ceftriaxone and Chloramphenicol is used to cure infection, however, their interaction has not been known exactly. **Objectives:** to evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *S. aureus* isolate of Diabetic gangrene. **Material and Methods:** The study was performed by using diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/mL, 15 µg/mL and 30 µg/mL, respectively were tested on *S. aureus* isolate of Diabetic gangrene. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. The interaction of antibacterial was analyzed using AZDAST method. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed antibacterial activity lower than single antibiotic. Antagonistic interaction is resulted by their combination. Ceftriaxone require the cell be growing and dividing in order to get bactericidal effect. Chloramphenicol inhibit growth of *S. aureus* and impairs bactericidal effect of Ceftriaxone. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single Ceftriaxone on *S. aureus* isolate of Gangrene diabetic. The type of interaction antibiotic is antagonistic

Keywords: Ceftriaxone; Chloramphenicol; Staphylococcus aureus isolate of Gangrene diabetic.

ABSTRAK

Latar Belakang: Gangrene diabetik merupakan salah satu komplikasi Diabetes melitus yang disebabkan oleh *S. aureus*. Kombinasi seftriakson dan kloramfenikol sering digunakan untuk pengobatan infeksi gangrene, meskipun interaksinya belum diketahui secara pasti. **Tujuan:** membuktikan potensi seftriakson, kloramfenikol dan kombinasinya pada isolat *S. aureus* dari gangrene diabetik. **Bahan dan Metode:** Penelitian dilakukan dengan metode difusi cakram dengan media Muller Hinton. Seftriakson, kloramfenikol dan kombinasinya dengan dosis 7,5 g/mL, 15 g/mL dan 30 g/mL, masing-masing diuji pada kultur *S. aureus* isolat gangrene diabetes. Efek antibakteri diamati dengan mengukur zona hambat pada kultur bakteri. Tipe interaksi dianalisa dengan metode AZDAST. Hasil penelitian diuji secara statistik dengan One Way ANOVA (p=0,05) dilanjutkan dengan uji Beda Nyata Terkecil (BNT). **Hasil:** Kombinasi seftriakson dan kloramfenikol menunjukkan efek antibakteri lebih rendah dibandingkan antibiotik tunggal seftriakson. Interaksi antagonis dihasilkan dari kombinasi antibiotic ini. Seftriakson memerlukan kondisi sel tumbuh dan membelah agar sebagai agen bakterisida. Sementara kloramfenikol menghambat pertumbuhan *S.aureus* dan mengganggu efek bakterisidal dari seftriakson bila dikombinasi. **Simpulan:** Kombinasi seftriakson dan kloramfenikol memiliki efek antibakteri yang lebih rendah dibandingkan dengan antibiotik seftriakson tunggal pada isolat *S. aureus* diabetes Gangrene. Tipe interaksi antibiotic bersifat antagonis.

Kata kunci: seftriakson; kloramfenikol; *staphylococcus aureus*, gangrene diabetik.

INTRODUCTION

Diabetic foot infection is a major cause of hospitalized patient in developing country (Złoch et al., 2021). In 2009, research at Makassar Hospital Indonesia indicated about 20,61% hospitalized patients were caused by diabetic foot infection (Rohmah, 2019). Severe diabetic foot infections and late handled can lead to gangrene diabetic (Aulia et al., 2019). Variety of bacteria causing infection of diabetic gangrene is a gram positive, gram negative and anaerobic bacteria (Prasetya et al., 2019). Research in the New England Deaconess Hospital indicated gangrene diabetic infection always caused more than 2 groups

of bacteria (Caroline, 2016). *Staphylococcus aureus*, *Streptococcus sp*, *Pseudomonas sp* are the major cause of gangrene infection (Yutaka, 2020).

One of pharmacological therapy for gangrene diabetic is antibiotics. The administration of antibiotics should be appropriate with the culture of gangrene bacteria on the infected wound. On the worsening of diabetic foot infections should be given antibiotics while waiting culture results. In cases of diabetic foot infection, combination of antibiotic is used to cure the infection (Caroline, 2016; Otoupal et al., 2021) Cephalosporin group are often used for such cases is Ceftriaxone (CTX) or Ceftazidim (Caroline, 2016; Jahani et al., 2017). The combination of antibiotics which include fixed combination has been proven and recognized as a fixed drug combination. Several combinations of antibiotics having bactericidal and bacteriostatic effect are not known exactly due to they are influenced by the dose (Navarro-Pérez et al., 2021). Theoretically, the combination of antibiotics is confirmed antagonist, however, it is still used because it is considered not clinically meaningful (Ocampo et al., 2014; Otoupal et al., 2021).

Treatment of diabetic foot infection using the combination of bactericidal antibiotic like Ceftriaxone (CTX) and bacteriostatic antibiotic like Chloramphenicol (CML) can produce antagonistic interactions based on theory (Navarro-Pérez et al., 2021; Złoch et al., 2021). Their interaction will reduce the efficacy of antibiotic. The study can be performed using diffusion method and type interaction with Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) (Ziaei-Darounkalaei et al., 2016). Based on explanation above, the objective of the study was to evaluate the combination effect of CTX and CML against *S. aureus* isolate of gangrene diabetic including the type of interaction.

MATERIAL AND METHODS

MATERIALS

S. aureus isolate gangrene diabetic (Dr. Saiful Anwar Hospital, Malang), Muller Hinton media (Sigma-Aldrich), CTX (Orchid Pharma), CML (Kimia Farma), Paper disk (Merck), Mc-Farland turbidity standard (Sigma-Aldrich).

METHODS

Bacteria media preparation

Muller Hinton media 3.8% was made by adding sterile distilled water and then boiled until complete dissolution. Muller Hinton media was poured into empty petri dish and left until hardened. Muller Hinton media has hardened put in an incubator with a temperature of 37°C for ± 24 hour to evaluate bacterial contaminants in the media that has been made. If a bacterial contaminant does not found therefore the media is ready to use.

Antibiotic Preparation

Both CTX and CML were each dissolved with sterile distilled water and then they were further dissolved to obtain 30 µg/ml, 15 µg/ml, 7.5 µg/ml. Furthermore, the combination of CTX and CML were made

to obtain three composition, the ratio CTX:CML are (C1) 30 : 7.5 µg/mL, (C2) 15 : 15 µg/mL, and (C3) 7.5 : 30 µg/mL. Paper disc was inserted to the antibiotic solution for preparation of antibacterial assay.

Antibacterial Assay (Referensi???)

S.aureus suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5 x 10^7 cells/ml) tubes. Next, it was diluted to obtain a final 5 x 10^6 cells/ml. Bacteria was sub-cultured on Muller Hinton media for bacterial propagation (Solomon and Isaac, 2018). The broth was inoculated by the 0.2 µg /ml by *S. aureus* and then added paper disk containing CTX, CML and its combination. Furthermore, the plates were incubated at 37 ± 1°C for 24 h and observed for colony growth. Clear zone was measured by a caliper showing zone of inhibition (ZOI). Antibacterial activity is classified based on Greenwood, 1995 in Ramadheni et al., 2018: none (less than 10 mm), weak (11-15 mm), moderate (16-20 mm), potent (more than 20 mm). Meanwhile, percent of inhibition was calculated based on formula

$$\text{Percent of inhibition} = \frac{\text{ZOI sample} - \text{ZOI control}}{1.5 \text{ ZOI potent (30 mm)}} \times 100\ \%$$

The IC_{50} value was calculated by linear regression curve fit using SPPS version 16.0

Analysis of antibacterial interaction

Interaction were analyzed using Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) method (Ziaei-Darounkalaei et al, 2016). Type of interaction are synergistic (AB >A&B and </> AA and/or BB), potentiation (one of A/B=0 and AB>A&B and >/< AA and/or BB), antagonistic (AB < A or B), additive (AB=AA and/or BB) and not distinguishable (AB = A or B)

Statistical analysis

The experimental results were replicated four times and the data are expressed as the mean ± SD. The data were tested using One Way ANOVA test and followed by Least Significant Difference (LSD, p=0,05) test.

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial activity of compound was showed by inhibition of bacterial growth. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* isolate of gangrene diabetic can be seen in the Table 1-2, and Figure 1-2.

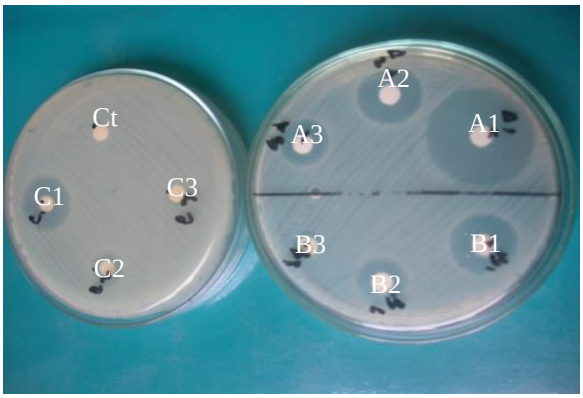

Figure 1. Inhibition zone diameter of Ceftriaxone, Chloramphenicol and their combination on *S. aureus*. isolate of gangrene diabetic (Ct=control, A1=CTX-30, A2=CTX-15, A3=CTX-7.5, B1=CML-7.5, B2=CML-15, B3=30, C1=CTX30-CML7.5, C2= CTX15-CML15, C3= CTX7.5-CML30)

Table 1. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* isolate of gangrene diabetic

No.	Group	n	Mean ± SD (mm)	Antibacterial effect*
1	Control (Ct)	4	6.00±0.00[a]	None
	CTX-30 (A1)	4	28.96±0.02[b]	Potent
	CML-7,5 (B3)	4	7.97±0.24[c]	None
	CTX-30:CML-7.5 (C1)	4	17.77±0.32[d]	Moderate
2	Control (Ct)	4	6.00±0.00[a]	None
	CTX-15 (A2)	4	21.00±0.05[b]	Potent
	CML-15 (B2)	4	12.37±0.49[c]	Weak
	CTX-15:CML-15 (C2)	4	14.03±0.22[c]	Weak
3	Control (Ct)	4	6.00±0.00[a]	None
	CTX-7,5 (A3)	4	11.88±0.00[b]	Weak
	CML-30 (B1)	4	17.98±0.05[c]	Moderate
	CTX-7,5:CML-30 (C3)	4	8.45±0.44[d]	None

Note :
a,b,c : different letter show the different effect (LSD, $p<0.05$ %)
* : antibacterial effect based on Greenwood classification, 1995 in Ramadheni et al (2018)

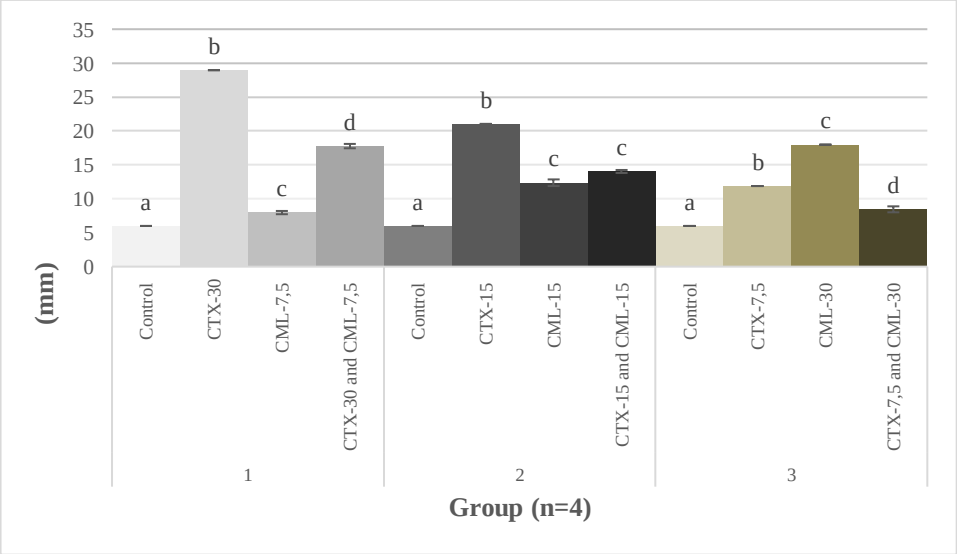

Figure 2. Inhibition zone diameter of CTX, CML and their combination on *S. aureus*

Table 2. Inhibitory concentration-50 (IC_{50}) of antibiotic on *S.aureus* isolate of gangrene diabetic

Antibiotic	Concentration (μg/mL)	n	Mean ± SD (mm)	% Inhibition	IC_{50} (μg/mL)
CTX	0	4	6.00±0.00[a]	0.00	
	7.5	4	11.88±0.00[b]	19.60	
	15	4	21.00±0.05[c]	50.00	18.32
	30	4	28.96±0.02[d]	76.53	
CML	0	4	6.00±0.00[a]	0.00	
	7.5	4	7.97±0.24[a]	6.56	37.17
	15	4	12.37±0.49[b]	21.23	
	30	4	17.98±0.05[c]	39.93	

Note :
a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

Based on these result, the first composition indicated a significant decrease of inhibition zone diameter on combination CTX-CML (30:7.5 μg/mL) compare to CTX 30 μg/mL. However, inhibition zone diameter of the combination was higher than CML 7.5 μg/mL and it has a moderate activity. CML showed a low antibacterial effect due to the dose used and the less sensitivity of them to *S. aureus* (Yutaka, 2020; Alsherbiny et al., 2021).

The second combination CTX-CML (15:15 μg/mL) indicated a significant reduce of inhibition zone diameter compare to CTX 15 μg/mL. On the other hand, inhibition zone diameter of the combination was higher than single CML 15 μg/mL and it has a weak activity. CTX more potent than CML at the same dose, it supported also with the percent inhibition and IC_{50} value which is showed at table 2. CTX has IC_{50} value lower than CML, it means CTX more potent to inhibit *S.aureus* growth compare to CML. For third combination CTX-CML (7.5:30 μg/mL) showed a significant decrease of inhibition zone diameter compare to both of single CTX 7.5 μg/mL and CML 30 μg/mL. This combination has lower antibacterial activity than two combination mentioned previous and according to Ramadheni et al., 2018, it does not have antibacterial effect. Three combination of CTX and CML are disadvantage since the potency of antibacterial decreased until it has no activity. The first combination has antibacterial effect stronger than two combination others. It is caused by the difference of composition bactericidal and bacteriostatic which is used for each combination. CTX has bactericidal effect whereas CML has bacteriostatic effect. When two antibiotics above are combined, the activity of bactericidal antibiotic will be inhibited by bacteriostatic antibiotic meaningful (Ocampo et al., 2014; Navarro-Pérez et al., 2021). CTX works through inhibiting the formation of cell wall, meanwhile CML blocking protein synthesis especially at ribosome sub unit 50S (Eyler and Shvets, 2019; Inayah et al., 2020). The combination with high dose of CTX and low dose of CML results a strong antibacterial effect on *S. aureus.*

Mixed infection caused by several groups of bacteria like in gangrene diabetic infection results the use of combination antibiotic (Caroline, 2016; Złoch et al., 2021). Antibiotic combination of CTX and CML

is used to increase efficacy on gangrene diabetic infection. The combination was selected to expand the spectrum of bacteria also (Kothari et al., 2022). Actually, the aims of antibacterial combination to increase potency, extend spectrum, reduce resistance and toxicity (Alsherbiny et al., 2021; Kothari et al., 2022). However, incorrect selection of antibacterial combination and the dose produce therapy failure (Gilbert et al., 2010; Parkunan et al., 2019).

Interaction of antibacterial CTX and CML

The interaction of CTX and CML is analyzed using AZDAST methods, the result can be seen at table 3

Table 3. Interaction of CTX and CML combination on *S. aureus*

Antibiotic	n	Mean ± SD (mm)	Antibacterial effect*	Type of interaction#
CTX-7.5	4	11.88±0.00[a]	Weak	
CML-7.5	4	7.97±0.24[b]	None	Antagonistic
CTX-7.5 + CML-7.5	4	9.02±0.13[b]	None	
CTX-7.5 + CTX-7.5	4	21.00±0.05[c]	Potent	
CML-7.5 + CML-7.5	4	12.37±0.49[a]	Weak	
CTX-15	4	21.00±0.05[a]	Potent	
CML-15	4	12.37±0.49[b]	Weak	Antagonistic
CTX-15 + CML-15	4	14.03±0.22[b]	Weak	
CTX-15 + CTX-15	4	28.96±0.02[c]	Potent	
CML-15 + CML-15	4	17.98±0.05[d]	Moderate	

Noted

a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

* : antibacterial effect based on Greenwood classification, 1995 in Ramadheni et al (2018)

: type of interaction based on AZDAST method

The combination produces antibacterial effect lower than single antibiotic due to antagonistic interaction between bactericidal antibiotic and bacteriostatic, therefore it reduces their potency as antibiotic. ß-lactam antibiotic like CTX require the cell be growing and dividing in order to have a bactericidal action. CML cause slow growth of *S. aureus* furthermore it impairs bactericidal effect of CTX (Ocampo et al., 2014; Navarro-Pérez et al., 2021). The combination CTX and CML result antagonistic interaction based on AZDAST method.

Conditions of bacterial growth affect the activity of bactericidal agents also. Conditions at the site of infection that favor no growth or slow growth of bacteria will disrupt bactericidal action. Thus, whether a bactericidal or a bacteriostatic effect is achieved can vary depending on not only the antibiotic, however, the organisms and the growth conditions also (Eyler and Shvets, 2019; Navarro-Pérez et al., 2021). Antibiotic was classified as bacteriostatic and bactericidal sketchily, bacteriostatic inhibits replication and growth of bacteria. Meanwhile bactericidal kill bacteria and reduce of bacteria number lived (Sari et al., 2018). Even though the classification is simple, in some case antibiotic is bacteriostatic for one organisms and are bactericidal to other organisms. For example, CML is bacteriostatic to gram-negative rods but it is bactericidal to *Pneumococus* sp (Sood, 2016; Lorenzo, 2019).

The experimental laboratory indicated the growth of bacteria was inhibited by bacteriostatic. Microorganisms can live although there were exposed by bacteriostatic drugs. On the other hand, giving of bactericidal drugs can kill bacteria and decrease of bacteria number lived (Otoupal et al., 2021). Mixed infection caused by several group bacterial such as gangrene diabetic induces the use of antibiotic combination (Caroline, 2016).

Antibiotics are effective drug in the therapy of infection diseases due to their potency to kill microorganisms. In many cases, antibiotic treatment requires controlling of dose to eradicate microorganisms furthermore the body can tolerate it (Paterson et al., 2016).

Selection of antibiotic for infection therapy requires the knowledge about microorganism identity and their sensitivity to antibiotic, place of infection, antibiotic safety, patient factor and cost (Gilbert et al., 2010). However, some seriously ill patient require empiric therapy that is giving antibiotic as soon as possible while waiting the culture results (Prasetya et al., 2019).

In addition, bactericidal activity is not an invariable property of an antibiotic, it can depend upon the organism and the growth conditions. For example, *S. aureus* is not killed by protein synthesis inhibitors like CML and Erythromycin (ERT), which are classical bacteriostatic agents having target at the ribosome (Eyler and Shvets, 2019; Schulte-Werning et al., 2021). The most of bacteria, *S. aureus* and *S. pneumoniae* are killed by cell wall inhibitors such as Penicillin and Vancomycin, as well as by the Fluoroquinolones, which are classical bactericidal agents (Navarro-Pérez et al., 2021). These agents *in vitro* do not produce 99.9% kill of Enterococci within the 24-hour time frame, therefore they are considered to be only bacteriostatic against this organism (Gilbert et al., 2010; Ocampo et al., 2014). The continue between bactericidal activity and bacteriostatic that varies depending on the antibiotic, the bacterial species or isolate, and growth conditions (Ocampo et al., 2014; Paolo et al., 2014).

CONCLUSION

Combination of CTX and CML has antibacterial effect lower than the single antibiotic groups on *S. aureus* isolate Gangrene diabetic and the type of interaction is antagonistic. The combination of high dose of CTX and low dose of CML shows antibacterial effect in the moderate category.

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No declared.

CONFLICT OF INTEREST

The author declared no conlict of interest

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The Combination Effect of Ceftriaxone and Chloramphenicol on *Staphylococcus aureus* Isolate of Diabetic Gangrene

(Efek Kombinasi Seftriakson dan Kloramfenikol pada Isolat Staphylococcus aureus dari Gangren Diabetik)

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ABSTRACT

Background: Diabetic gangrene is a complication of Diabetes mellitus caused by *Staphylococcus aureus*. The combination of Ceftriaxone and Chloramphenicol is often used to cure gangrene infection, even though, they produce antagonist interaction based on theory. **Objectives:** To evaluate the potency of Ceftriaxone, Chloramphenicol and its combination on *Staphylococcus aureus* isolate of Diabetic gangrene. **Material and Methods:** The research was done by using disc diffusion methods with Muller Hinton media. Ceftriaxone, Chloramphenicol and its combination dose of 7,5 µg/ml, 15 µg/ml and 30 µg/ml, respectively were tested on *Staphylococcus aureus* culture taken form the diabetic gangrene patients. Antibacterial effect was observed by measuring inhibition zone on bacteria culture. Type of interaction was analyzed by Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) method. The results of study were tested statistically with One Way ANOVA (p=0.05) followed by Least Significant Difference (LSD) test. **Results:** The combination of Ceftriaxone and Chloramphenicol showed an antibacterial effect lower than Ceftriaxone. ß-lactam antibiotic like Ceftriaxone require the cell be growing and dividing in order to have a bactericidal action. Meanwhile, Chloramphenicol causes a slow growth of *Staphylococcus aureus* and impairs bactericidal effect of Ceftriaxone if they are combined. **Conclusions:** Ceftriaxone and Chloramphenicol combination has lower antibacterial effect than the single antibiotic groups on *Staphylococcus aureus* isolate of Gangrene diabetic and the type of interaction is antagonistic.

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ABSTRAK

Latar Belakang: *Gangrene diabetik* merupakan salah satu komplikasi Diabetes melitus yang disebabkan oleh *S. aureus*. Kombinasi seftriakson dan kloramfenikol sering digunakan untuk pengobatan infeksi gangrene, meskipun interaksinya belum diketahui secara pasti. Tujuan: membuktikan potensi seftriakson, kloramfenikol dan kombinasinya pada isolat *S. aureus* dari gangrene diabetik. Bahan dan Metode: Penelitian dilakukan dengan metode difusi cakram dengan media Muller Hinton. Seftriakson, kloramfenikol dan kombinasinya dengan dosis 7,5 g/mL, 15 g/mL dan 30 g/mL, masing-masing diuji pada kultur *S. aureus* isolat gangrene diabetes. Efek antibakteri diamati dengan mengukur zona hambat pada kultur bakteri. Tipe interaksi dianalisa dengan metode Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST). Hasil penelitian diuji secara statistik dengan One Way ANOVA (p=0,05) dilanjutkan dengan uji Beda Nyata Terkecil (BNT). Hasil: Kombinasi seftriakson dan kloramfenikol menunjukkan efek antibakteri lebih rendah dibandingkan antibiotik tunggal seftriakson. Interaksi antagonis dihasilkan dari kombinasi antibiotik ini. Seftriakson memerlukan kondisi sel tumbuh dan membelah agar berfungsi sebagai agen bakterisida. Sementara kloramfenikol menghambat pertumbuhan *S.aureus* dan mengganggu efek bakterisidal dari seftriakson bila dikombinasi. Kesimpulan: Kombinasi seftriakson dan kloramfenikol memiliki efek antibakteri yang lebih rendah dibandingkan dengan antibiotik seftriakson tunggal pada isolat *S. aureus* diabetes Gangrene. Tipe interaksi antibiotik bersifat antagonis.

Kata kunci: Seftriakson; Kloramfenikol; *Staphylococcus aureus*, gangrene diabetik.

INTRODUCTION

Diabetic foot infection is a major cause of hospitalized patient in developing country (Złoch et al., 2021). In 2009, research at Makassar Hospital Indonesia indicated about 20,61% hospitalized patients were caused by diabetic foot infection (Rohmah, 2019). Severe diabetic foot infections and late handled can lead to gangrene diabetic (Aulia et al., 2019). Variety of bacteria causing infection of diabetic gangrene is a gram positive, gram negative and anaerobic bacteria (Prasetya et al., 2019). Research in the New England Deaconess Hospital indicated gangrene diabetic infection always caused more than 2 groups of bacteria (Caroline, 2016). *Staphylococcus aureus*, *Streptococcus sp*, *Pseudomonas sp* are the major cause of gangrene infection (Yutaka, 2020).

One of pharmacological therapy for gangrene diabetic is antibiotics. The administration of antibiotics should be appropriate with the culture of gangrene bacteria on the infected wound. On the worsening of diabetic foot infections should be given antibiotics while waiting culture results. In cases of diabetic foot infection, combination of antibiotic is used to cure the infection (Caroline, 2016; Otoupal et al., 2021) Cephalosporin group are often used for such cases is Ceftriaxone (CTX) or Ceftazidim (Caroline, 2016; Jahani et al., 2017). The combination of antibiotics which include fixed combination has been proven and recognized as a fixed drug combination. Several combinations of antibiotics having bactericidal and bacteriostatic effect are not known exactly due to they are influenced by the dose (Navarro-Pérez et al., 2021). Theoretically, the combination of antibiotics is confirmed antagonist, however, it is still used because it is considered not clinically meaningful (Ocampo et al., 2014; Otoupal et al., 2021).

Treatment of diabetic foot infection using the combination of bactericidal antibiotic like Ceftriaxone (CTX) and bacteriostatic antibiotic like Chloramphenicol (CML) can produce antagonistic interactions

based on theory (Navarro-Pérez et al., 2021; Złoch et al., 2021). Their interaction will reduce the efficacy of antibiotic. The study can be performed using diffusion method and type interaction with Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) (Ziaei-Darounkalaei et al., 2016). Based on explanation above, the objective of the study was to evaluate the combination effect of CTX and CML against *S. aureus* isolate of gangrene diabetic including the type of interaction.

MATERIAL AND METHODS

MATERIALS

S. aureus isolate of gangrene diabetic was obtained from Dr. Saiful Anwar Hospital, Malang, Muller Hinton media (Sigma-Aldrich), Ceftriaxone (Orchid Pharma), Chloramphenicol (Kimia Farma), Paper disk (Merck), and Mc-Farland turbidity standard (Sigma-Aldrich).

METHODS

Bacteria media preparation

Muller Hinton media 3.8% was made by adding sterile distilled water and then boiled until complete dissolution. Muller Hinton media was poured into empty petri dish and left until hardened. Muller Hinton media has hardened put in an incubator with a temperature of 37°C for ± 24 hour to evaluate bacterial contaminants in the media that has been made. If a bacterial contaminant does not found therefore the media is ready to use.

Antibiotic Preparation

Both CTX and CML were each dissolved with sterile distilled water and then they were further dissolved to obtain 30 µg/ml, 15 µg/ml, 7.5 µg/ml. Furthermore, the combination of CTX and CML were made to obtain three composition, the ratio CTX:CML are (C1) 30 : 7.5 µg/mL, (C2) 15 : 15 µg/mL, and (C3) 7.5 : 30 µg/mL. Paper disc was inserted to the antibiotic solution for preparation of antibacterial assay.

Antibacterial Assay

S.aureus suspension was prepared and adjusted by comparison against 0.5 Mc-Farland turbidity standard (5 x 10^7 cells/ml) tubes. Next, it was diluted to obtain a final 5 x 10^6 cells/ml. Bacteria was sub-cultured on Muller Hinton media for bacterial propagation (Solomon and Isaac, 2018). The broth was inoculated by the 0.2 µg /ml by *S. aureus* and then added paper disk containing CTX, CML and its combination. Furthermore, the plates were incubated at 37 ± 1°C for 24 h and observed for colony growth. Clear zone was measured by a caliper showing zone of inhibition (ZOI). Antibacterial activity is classified based on Greenwood, 1995 in Ramadheni et al., 2018: none (less than 10 mm), weak (11-15 mm), moderate (16-20 mm), potent (more than 20 mm). Meanwhile, percent of inhibition was calculated based on formula

$$\text{Percent of inhibition} = \frac{\text{ZOI sample} - \text{ZOI control}}{1.5 \text{ ZOI potent (30 mm)}} \times 100\ \%$$

The IC_{50} value was calculated by linear regression curve fit using SPPS version 16.0

Analysis of antibacterial interaction

Interaction were analyzed using Ameri-Ziaei Double Antibiotic Synergism Test (AZDAST) method (Ziaei-Darounkalaei et al, 2016). Type of interaction are synergistic (AB >A&B and </> AA and/or BB), potentiation (one of A/B=0 and AB>A&B and >/< AA and/or BB), antagonistic (AB < A or B), additive (AB=AA and/or BB) and not distinguishable (AB = A or B).

Statistical analysis

The experimental results were replicated four times and the data are expressed as the mean ± SD. The data were tested using One Way ANOVA test and followed by Least Significant Difference (LSD, p=0,05) test.

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial activity of compound was showed by inhibition of bacterial growth. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* isolate of gangrene diabetic can be seen in the Table 1-2, and Figure 1-2.

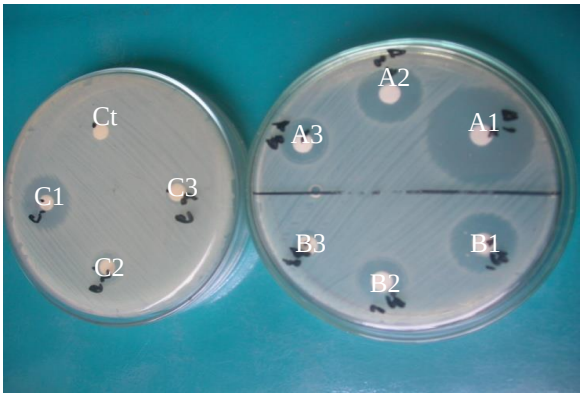

Figure 1. Inhibition zone diameter of Ceftriaxone, Chloramphenicol and their combination on *S. aureus.* isolate of gangrene diabetic (Ct=control, A1=CTX-30, A2=CTX-15, A3=CTX-7.5, B1=CML-7.5, B2=CML-15, B3=30, C1=CTX30-CML7.5, C2= CTX15-CML15, C3= CTX7.5-CML30).

Table 1. Inhibition zone diameter of CTX, CML and their combination on *S. aureus* isolate of gangrene diabetic

No.	Group	n	Mean ± SD (mm)	Antibacterial effect*
1	Control (Ct)	4	6.00±0.00[a]	None
	CTX-30 (A1)	4	28.96±0.02[b]	Potent
	CML-7,5 (B3)	4	7.97±0.24[c]	None
	CTX-30:CML-7.5 (C1)	4	17.77±0.32[d]	Moderate
2	Control (Ct)	4	6.00±0.00[a]	None
	CTX-15 (A2)	4	21.00±0.05[b]	Potent
	CML-15 (B2)	4	12.37±0.49[c]	Weak
	CTX-15:CML-15 (C2)	4	14.03±0.22[c]	Weak
3	Control (Ct)	4	6.00±0.00[a]	None
	CTX-7,5 (A3)	4	11.88±0.00[b]	Weak
	CML-30 (B1)	4	17.98±0.05[c]	Moderate
	CTX-7,5:CML-30 (C3)	4	8.45±0.44[d]	None

a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

* : antibacterial effect based on Greenwood classification, 1995 in Ramadheni et al (2018)

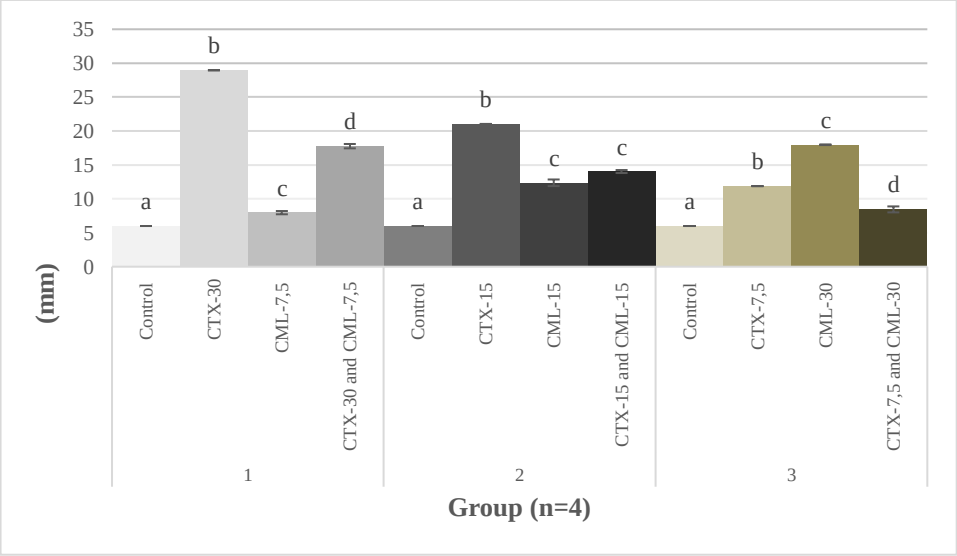

Figure 2. Inhibition zone diameter of CTX, CML and their combination on *S. aureus*

Table 2. Inhibitory concentration-50 (IC_{50}) of antibiotic on *S.aureus* isolate of gangrene diabetic

Antibiotic	Concentration (μg/mL)	n	Mean ± SD (mm)	% Inhibition	IC_{50} (μg/mL)
CTX	0	4	6.00±0.00[a]	0.00	
	7.5	4	11.88±0.00[b]	19.60	
	15	4	21.00±0.05[c]	50.00	18.32
	30	4	28.96±0.02[d]	76.53	
CML	0	4	6.00±0.00[a]	0.00	
	7.5	4	7.97±0.24[a]	6.56	37.17
	15	4	12.37±0.49[b]	21.23	
	30	4	17.98±0.05[c]	39.93	

a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

Based on these result, the first composition indicated a significant decrease of inhibition zone diameter on combination CTX-CML (30:7.5 μg/mL) compare to CTX 30 μg/mL. However, inhibition zone diameter of the combination was higher than CML 7.5 μg/mL and it has a moderate activity. CML showed a low antibacterial effect due to the dose used and the less sensitivity of them to *S. aureus* (Yutaka, 2020; Alsherbiny et al., 2021).

The second combination CTX-CML (15:15 μg/mL) indicated a significant reduce of inhibition zone diameter compare to CTX 15 μg/mL. On the other hand, inhibition zone diameter of the combination was higher than single CML 15 μg/mL and it has a weak activity. CTX more potent than CML at the same dose, it supported also with the percent inhibition and IC_{50} value which is showed at table 2. CTX has IC_{50} value lower than CML, it means CTX more potent to inhibit *S.aureus* growth compare to CML.

For third combination CTX-CML (7.5:30 μg/mL) showed a significant decrease of inhibition zone diameter compare to both of single CTX 7.5 μg/mL and CML 30 μg/mL. This combination has lower antibacterial activity than two combination mentioned previous and according to Ramadheni et al., 2018, it does not have antibacterial effect. Three combination of CTX and CML are disadvantage since the potency of antibacterial decreased until it has no activity. The first combination has antibacterial effect stronger than two combination others. It is caused by the difference of composition bactericidal and bacteriostatic which is used for each combination. CTX has bactericidal effect whereas CML has bacteriostatic effect. When two antibiotics above are combined, the activity of bactericidal antibiotic will be inhibited by bacteriostatic antibiotic meaningful (Ocampo et al., 2014; Navarro-Pérez et al., 2021). CTX works through inhibiting the formation of cell wall, meanwhile CML blocking protein synthesis especially at ribosome sub unit 50S (Eyler and Shvets, 2019; Inayah et al., 2020). The combination with high dose of CTX and low dose of CML results a strong antibacterial effect on *S. aureus.*

Mixed infection caused by several groups of bacteria like in gangrene diabetic infection results the use of combination antibiotic (Caroline, 2016; Złoch et al., 2021). Antibiotic combination of CTX and CML

is used to increase efficacy on gangrene diabetic infection. The combination was selected to expand the spectrum of bacteria also (Kothari et al., 2022). Actually, the aims of antibacterial combination to increase potency, extend spectrum, reduce resistance and toxicity (Alsherbiny et al., 2021; Kothari et al., 2022). However, incorrect selection of antibacterial combination and the dose produce therapy failure (Gilbert et al., 2010; Parkunan et al., 2019).

Interaction of antibacterial CTX and CML

The interaction of CTX and CML is analyzed using AZDAST methods, the result can be seen at table 3

Table 3. Interaction of CTX and CML combination on *S. aureus*

Antibiotic	n	Mean ± SD (mm)	Antibacterial effect*	Type of interaction#
CTX-7.5	4	11.88±0.00[a]	Weak	
CML-7.5	4	7.97±0.24[b]	None	Antagonistic
CTX-7.5 + CML-7.5	4	9.02±0.13[b]	None	
CTX-7.5 + CTX-7.5	4	21.00±0.05[c]	Potent	
CML-7.5 + CML-7.5	4	12.37±0.49[a]	Weak	
CTX-15	4	21.00±0.05[a]	Potent	
CML-15	4	12.37±0.49[b]	Weak	Antagonistic
CTX-15 + CML-15	4	14.03±0.22[b]	Weak	
CTX-15 + CTX-15	4	28.96±0.02[c]	Potent	
CML-15 + CML-15	4	17.98±0.05[d]	Moderate	

a,b,c : different letter show the different effect (LSD, $p<0.05$ %)

* : antibacterial effect based on Greenwood classification, 1995 in Ramadheni et al (2018)

: type of interaction based on AZDAST method

The combination produces antibacterial effect lower than single antibiotic due to antagonistic interaction between bactericidal antibiotic and bacteriostatic, therefore it reduces their potency as antibiotic. ß-lactam antibiotic like CTX require the cell be growing and dividing in order to have a bactericidal action. CML cause slow growth of *S. aureus* furthermore it impairs bactericidal effect of CTX (Ocampo et al., 2014; Navarro-Pérez et al., 2021). The combination CTX and CML result antagonistic interaction based on AZDAST method.

Conditions of bacterial growth affect the activity of bactericidal agents also. Conditions at the site of infection that favor no growth or slow growth of bacteria will disrupt bactericidal action. Thus, whether a bactericidal or a bacteriostatic effect is achieved can vary depending on not only the antibiotic, however, the organisms and the growth conditions also (Eyler and Shvets, 2019; Navarro-Pérez et al., 2021). Antibiotic was classified as bacteriostatic and bactericidal sketchily, bacteriostatic inhibits replication and growth of bacteria. Meanwhile bactericidal kill bacteria and reduce of bacteria number lived (Sari et al., 2018). Even though the classification is simple, in some case antibiotic is bacteriostatic for one organisms and are bactericidal to other organisms. For example, CML is bacteriostatic to gram-negative rods but it is bactericidal to *Pneumococcus* sp (Sood, 2016; Lorenzo, 2019).

The experimental laboratory indicated the growth of bacteria was inhibited by bacteriostatic. Microorganisms can live although there were exposed by bacteriostatic drugs. On the other hand, giving of bactericidal drugs can kill bacteria and decrease of bacteria number lived (Otoupal et al., 2021). Mixed infection caused by several group bacterial such as gangrene diabetic induces the use of antibiotic combination (Caroline, 2016).

Antibiotics are effective drug in the therapy of infection diseases due to their potency to kill microorganisms. In many cases, antibiotic treatment requires controlling of dose to eradicate microorganisms furthermore the body can tolerate it (Paterson et al., 2016).

Selection of antibiotic for infection therapy requires the knowledge about microorganism identity and their sensitivity to antibiotic, place of infection, antibiotic safety, patient factor and cost (Gilbert et al., 2010). However, some seriously ill patient require empiric therapy that is giving antibiotic as soon as possible while waiting the culture results (Prasetya et al., 2019).

In addition, bactericidal activity is not an invariable property of an antibiotic, it can depend upon the organism and the growth conditions. For example, *S. aureus* is not killed by protein synthesis inhibitors like CML and Erythromycin (ERT), which are classical bacteriostatic agents having target at the ribosome (Eyler and Shvets, 2019; Schulte-Werning et al., 2021). The most of bacteria, *S. aureus* and *S. pneumoniae* are killed by cell wall inhibitors such as Penicillin and Vancomycin, as well as by the Fluoroquinolones, which are classical bactericidal agents (Navarro-Pérez et al., 2021). These agents *in vitro* do not produce 99.9% kill of Enterococci within the 24-hour time frame, therefore they are considered to be only bacteriostatic against this organism (Gilbert et al., 2010; Ocampo et al., 2014). The continue between bactericidal activity and bacteriostatic that varies depending on the antibiotic, the bacterial species or isolate, and growth conditions (Ocampo et al., 2014; Paolo et al., 2014).

CONCLUSION

Combination of CTX and CML has antibacterial effect lower than the single antibiotic groups on *S. aureus* isolate Gangrene diabetic and the type of interaction is antagonistic. The combination of high dose of CTX and low dose of CML shows antibacterial effect in the moderate category.

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No declared.

CONFLICT OF INTEREST

The author declared no conlict of interest

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