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Research Article

# The Antibacterial Activity of Gentamicin Sulphate in NaCl and Dextrose-NaCl Infusion against *Escherichia coli* Atcc 25922 and *Staphylococcus aureus* Atcc 29213

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**ABSTRACT**

Gentamicin sulphate mixed into the fluid infusion of NaCl and dextrose-NaCl is the process of intravenous admixtures, where knowledge about sterility, physicochemistry and stability properties of drugs, incompatible of drugs and the risk of exposure are matter to be considered. An already reconstituted drug has a time limit of stability and the long-time mixing will decrease its activity in inhibiting bacteria. The study aims was to determined the antibacterial effect of the infusion of 0.9% NaCl and 5% dextrose-0.225% NaCl against Escherichia coli and Staphylococcus aureus, the influence of time variation of the use of gentamicin sulphate injection, and the effect of interaction of infusion and time of use towards its activity on bacteria. The laboratory experiment research method was done, which included mixing sterile preparation of gentamicin sulphate injection into NaCl and dextrose-NaCl infusion in LAF, tested antibacterial activity against Escherichia coli and Staphylococcus aureus and data analyzed with ANOVA. The results showed that the type of fluid infusion was only influential as antibacterial activity against Staphylococcus aureus but not against Escherichia coli. On the other hand, the use of time variation was effective on gentamicin sulphate activity against both bacteria. Also, the interaction of fluid infusion with time of use has no effect on its activity on bacteria.

**Keywords**: *5% dextrose, 0.225% NaCl, 0.9% NaCl, Escherichia coli, gentamicin sulphate injection, Staphylococcus aureus.*

## INTRODUCTION

Infectious diseases are a major issue and still ranks high among health issues in developing countries including Indonesia, which has also contributed significantly to the number of ilnesses and deaths[1]. One of the most deadly infectious diseases in Indonesia is diseases of the gastrointestinal tract. In addition to causing death, this disease is also a cause of nosocomial infections[2]. The spread of the disease can occur from one person to another, or from animal to human. The infection of the digestive tract, among others, can be caused by *Escherichia coli* and *Staphylococcus aureus* bacteria[3].

Diseases caused by bacteria or pathogenic microorganisms can be cured with antibiotic therapy[4,5]. Treatment is generally in the form of a aminoglycoside plus ampicillin[6]. New antobiotics or modification of existing antibiotics are needed to respond to the development of bacteria resistant to antibiotics. Gentamicin sulphate has the ability to kill *Escherichia coli* bacteria[7]. Gentamicin sulphate antibiotic is the most widely used due to the relatively broad spectrum of its antibacterial properties and resistance to heat[8,9]. These antibiotics work by inhibiting protein synthesis mechanism[8]. Antibiotics are widely used as chemotherapy drugs to prevent and treat bacterial infections[10]. Gentamicin is commonly used to treat severe infections caused by *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella* spp., *Enterobacter* spp., *Serratia* spp and for prophylaxis against endocarditis mainly[11].

Gentamicin sulphate is formulated in the form of dry injection due to its instability as a solution. According to the Indonesian Ministry of Health, gentamicin sulphate is compatible in 0.9% NaCl, 5% dextrose, 10% dextrose, 5% dextrose-0.225% NaCl, ringer's, and ringer's lactate solutions[12]. Gentamicin sulphate mixed into the fluid infusion of NaCl and dextrose-NaCl is the process of mixing intravenous infusion. Mixing the intravenous infusion should be performed by a pharmacist in Hospital Pharmacy Installation with sufficicient background knowledge of, among others, sterility, physicochemistry and stability properties of drugs, incompatibility of drug, and drug exposure hazards[13,14]. The place and long storage also affect the stability of the drug. A drug that is already reconstituted has a time limit of stability and the long-time mixing will decrease its activity in inhibiting bacteria[15].

According to Monica et al, the use of ringer's lactate solution and ringer's dextrose in mixing of gentamicin sulphate injection has an effect on antibacterial activity of

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*Escherichia coli* and *Staphylococcus aureus*[16]. Based on research carried out by Setyarini et al, the type of infusion has a significant effect in decreasing gentamicin sulphate injection. From these results, it is advisable to conduct further research on the activity of gentamicin sulphate mixed into other types of fluid infusion and against other bacteria[17].

## MATERIALS AND METHODS

*Tools*

Tools used in this research were Laminar Air flow cabinet (Esco®), autoclave (GEA®), incubators (Memmert®), oven (Memmert 854®), a sterile petri dish (Pyrex®), Erlenmeyer (Pyrex®), sterile cotton wool, sterile gauze, sterile volume pipette, sterile mikropipet (Eppendorf®), Spirit lamp, sterile reaction tube (Pyrex®), reaction tube rack, perforator, sterile syringe (Terumo®Syringe), and sterile ose.

*Materials*

The materials used in this research were gentamicin sulphate injection (Sagestam®) Batch number SH3414, infusion of 0.9% NaCl (Otsu-NS®) Batch number 46I88A, 5% dextrose-0.225% NaCl (Otsu D5,1/4NS®) Batch number 46A97A, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, nutrient agar (NA) (Oxoid®), fluid thioglycolate medium (FTM) (Oxoid®), tryptic soy broth (TSB) (Oxoid®), tryptic soy agar (TSA) (Oxoid®).

*Methods*

The research was a laboratory experiment with the following stages:

*Sterility Testing Laminar Workspace Air Flow (LAF)*

LAF space cleaned with alcohol 70%. Then, a UV lamp and ablower were turned on for 1 hour. The 20 mL sterile TSA media was placed into the sterile petri dish. Afterwards, the petri dish was opened and kept in the LAF workspace for 15 minutes. Subsequently petri dish were incubated during 18-24 hours at 37ºC[13].

*Evaluation of medium to test the sterility of the sample*

Fertility test media

A total of 15 mL of FTM and TSB media were inserted into test tubes. Then, the bacteria *E. coli* and *S. aureus* are inoculated into their respective media. Afterwards, bacterial inoculation in FTM medium was incubated for 7 days at a temperature of 30-35ºC, and fungi inoculation in TSB medium was incubated for 7 days at a temperature of 20-25ºC. Then, observations of turbidity on media was conducted[18].

*Test the effectiveness of media*

A total of 15 mL of FTM and TSB media were inserted into test tubes. Then, the bacteria *E. coli* and *S. aureus* were inoculated into their respective media and added to 1 mL of the test sample (gentamicin sulphate mixing results in NaCl and dextrose-NaCl solutions). Furthermore, bacterial inoculation in FTM media incubated for 7 days at a temperature of 30-35ºC and inoculation of bacteria on the media they will be incubated for 7 days at a temperature of 20-25ºC. After it conducted observations of turbidity on media[18].

*The mixing of sterile preparations*

Injection solution of gentamicin sulphate was mixed in NaCl and dextrose- NaCl solutions in LAF[13].

*Evaluation of intravenous gentamicin sulphate dosage*

*Organoleptic*

Organoleptic observations were performed on injection of gentamicin sulphate mixed with fluid infusion. Observed changes in the color, odor, clarity, and form of preparation that occurs[18].

*Sterility test*

The sterility test was performed by taking 1 mL of the test sample and put into test tubes 15 mL for each media FTM and TSB. Then, the FTM media was incubated at 30-35ºC and TSB media was incubated at a 20-25ºC. To observe the presence of bacterial growth, observations of turbidity samples were conducted over a period of 14 days[18].

*Confirmation test the bacteria Escherichia coli and Staphylococcus aureus*

*Biochemical Test*

Biochemical tests performed include a test fermentation of carbohydrates (sucrose, glucose, mannose, maltose, lactose), Voges-Prokauer test, methyl red test, and indol test. This test is done by inserting 1 ose of bacteria *E. coli* and *S. aureus* into each medium. After that incubated during 18-24 hours at 37ºC. Motility test was done by stabbing 1 ose of bacteria *E. coli* and *S. aureus* into the test medium. Then incubated during 18-24 hours at 37ºC. TSIA, urease, and citrate tests were carried out by scraping each *E. coli* and *S. aureus* on a sloping agar medium incubated for 18-24 hours at 37ºC[19,20].

*Gram Staining*

Gram staining was done by flooding the bacterial suspension with violet gentiant for 1 minute. Then, it was rinsed with distilled water. Subsequently, the dye was flooded with Lugol's solution for 2 minutes. Then, it was rinsed with distilled water. After that, it was rinsed again with 95% alcohol and distilled water. The banding solution is flooded again with a counter dye (water for 30 seconds). Excess color is removed and rinsed with distilled water and then dried with filter paper. After that, a small amount of emersion oil was applied to the preparation, then examined under a microscope[21].

*Gentamicin sulphate injection activity test in infusion preparations against Escherichia coli and Staphylococcus aureus bacteria*

Activity testing was performed using diffusion method for perforation technique. Fifty µL suspense bacteria (each *E. coli* and *S. aureus*) were mixed with 50 mL of a solution of sterile MHA, then homogenized and left until solidified. Holes were made in the media by using a sterile perforator and in each of these holes filled as much as 50 µL with gentamicin sulphate injection dosage that has been mixed with NaCl and dextrose-NaCl solutions (each with its own variation of the time of use for 0, 30, 60, 90, 120 minutes) using micropipette. After that, it was incubated during 18-24 hours at 37ºC. Next, the drag zone was formed by measuring the diameter using a caliper[22].

*Data Analysis*

The data obtained were analyzed using analysis of variance (ANOVA) with value of α was 5% and followed by Tukey test.[23] There were three hypotheses, namely:

Table 1: Fertility test FTM and TSB media.

| Time (Day) | FTM | | TSB |
|---|---|---|---|
| | *Escherichia coli* | *Staphylococcus aureus* | *Candida albicans* |
| 1 | + | + | + |
| 2 | + | + | + |
| 3 | + | + | + |
| 5 | + | + | + |
| 7 | + | + | + |

Notes: + Turbid (the growth of microorganisms)
- Not turbid (no growth of microorganisms)

Table 2: Effectiveness test FTM and TSB media with mixture of gentamicin sulphate in 0.9% NaCl solution.

| Time (Day) | FTM | | TSB |
|---|---|---|---|
| | *Escherichia coli* | *Staphylococcus aureus* | *Candida albicans* |
| 1 | + | + | - |
| 2 | + | + | - |
| 3 | + | + | - |
| 5 | + | + | + |
| 7 | + | + | + |

Notes: + Turbid (the growth of microorganisms)
- Not turbid (no growth of microorganisms)

Table 3: Effectiveness test media FTM and TSB with mixture of gentamicin sulphate in 5% dextrose-0.9% NaCl solutions.

| Time (Day) | FTM | | TSB |
|---|---|---|---|
| | *Escherichia coli* | *Staphylococcus aureus* | *Candida albicans* |
| 1 | + | + | - |
| 2 | + | + | - |
| 3 | + | + | + |
| 5 | + | + | + |
| 7 | + | + | + |

Notes: + Turbid (the growth of microorganisms)
- Not turbid (no growth of microorganisms)

*Variation of Infusion*

$H_0$: A mixture of gentamicin sulphate in fluids infusion has not effect to the activity of gentamicin sulphate

$H_1$: A mixture of gentamicin sulphate in fluids infusion effects to the activity of gentamicin sulphate

*Variation of time*

$H_0$ : variation of the time use of gentamicin sulphate mixture in the fluid infusion has not effect to the activity of gentamicin sulphate

$H_1$ : variation of the time use of gentamicin sulphate mixture in the fluid infusion effects to the activity of gentamicin sulphate

The interaction between fluid infusion and time of use

$H_0$: the interaction between fluid infusion and time variation of use has not effect to the activity of gentamicin sulphate.

$H_1$: the interaction between fluid infusion and time variation of use effect to the activity of gentamicin sulphate.

## RESULTS AND DISCUSSION

*Testing of Sterility Workspace Laminar Air Flow (LAF)*

Testing of sterility LAF, it was found that there were two colonies growing on TSA media. It still qualified because according to 4th Indonesian Pharmacopeia, the number allowed to grow in the LAF work space was less than 100 colonies.

*Media Evaluation for Sterility Sample Testing*

Media evaluation for sample sterility testing included fertility testing and effectiveness. The results of each test can be seen in Table 1 for fertility testing as well as Table 2 and Table 3 for effectiveness testing.

Media fertility testing was carried out to test the media used to grow bacteria or not. The media that have been inoculated microorganisms can be used as growth medium because it gave turbidity (+) result after incubation for 7 days at the appropriate temperature. Turbidity in both media indicates that FTM showed to be suitable for *Escherichia coli* and *Staphylococcus aureus* growth, while TSB media csuitable for *Candida albicans* growth. The result of fertility testing can be concluded that FTM and TSB media used were fertile (can be used as media for microorganism growth).

Media effectiveness testing was conducted to test whether the media used remained capable of growing bacteria after the addition of the sample. The FTM that has been mixed with the test sample both in 0.9% NaCl solution as well as 5% dextrose-0.225% NaCl showed good turbidity after incubation for 7 days. However, in the TSB medium there was a difference in turbidity time between the mixture of the test sample in 0.9% NaCl and mixture of 5% dextrose-0.225% NaCl, it was because the fungus will grow after the 3rd day of incubation. Based on the result of the research, it could be concluded that FTM and TSB media were still able to grow microorganism after the addition of test sample (mixture of gentamicin sulphate in infusion of 0.9% NaCl and 5% dextrose-0.225% NaCl).

*Mixing Sterile preparations*

The mixing process of injection solution of gentamicin with infusion fluid was aseptic in LAF and near the fire. The test sample was the result of mixing 2 mL of injection solution of gentamicin sulphate ad 100 mL of 0.9% NaCl solution and mixing 2 mL of injection solution gentamycin sulfate ad 100 mL of 5% dextrose-0.225% NaCl. Mixing was done at the time variation of 0, 30, 60, 90, and 120 minutes.

*Evaluation of Gentamicin Sulphate Infusion Dosage*

After mixing the test preparation then the evaluation of the preparation either organoleptically or sterility. The results of evaluation of gentamicin sulphate infusion preparation can be seen in Table 4 and Table 5.

The result showed that the evaluation on the two test samples performed organoleptically in the form of liquid, colorless, clear and no smell, the result has fulfilled the sterile injection supply requirements based on 4th Indonesian Pharmacopoeia.

The sterility test showed that the FTM and TSB media were not turbid (-) after incubation for 14 days. The result of sterility testing of mixing gentamicin sulfate in both infusion fluid of 0.9% NaCl and in infusion fluids of 5%

Table 4: Organoleptic testing of mixing results of gentamicin sulphate in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions.

| Observation Minute to- | Observational Characteristics | | | |
|---|---|---|---|---|
| | Form | Color | Clarity | Smell |
| 0 | Liquid | Colorless | Clear | No smell |
| 30 | Liquid | Colorless | Clear | No smell |
| 60 | Liquid | Colorless | Clear | No smell |
| 90 | Liquid | Colorless | Clear | No smell |
| 120 | Liquid | Colorless | Clear | No smell |

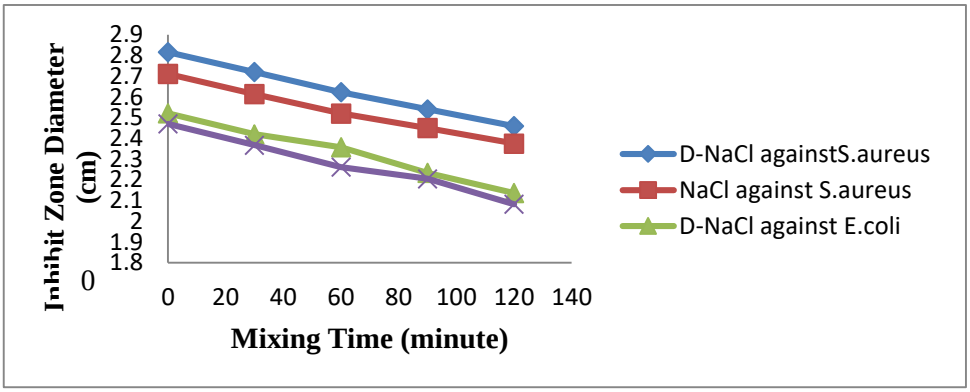


Figure 1: Test results of gentamicin sulphate activity in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions against *Escherichia coli* and *Staphylococcus aureus* bacteria.

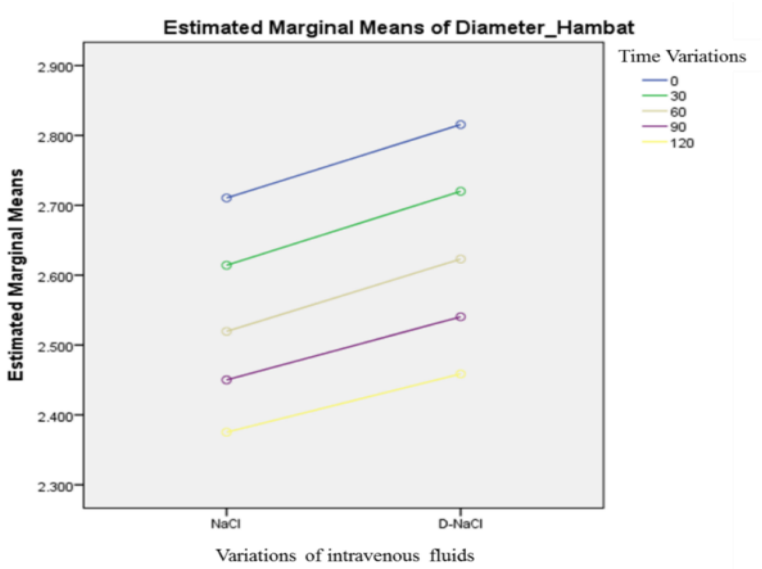


Figure 2: Inflows fluid infusion curve of in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions against *S. aureus*.

dextrose-0.225% NaCl was qualified in accordance with the provisions in 4th Indonesian Pharmacopoeia.

*Test for bacterial Escherichia coli and Staphylococcus aureus confirmation*

Bacterial confirmation tests performed include biochemical test and Gram staining test. Biochemical test results of both bacteria can be seen in Table 6.

The results showed that the test bacteria used were indeed the bacteria *E. coli* and *S. aureus*. In addition, other tests conducted in the bacterial affirmation are Gram stain. Gram staining on *E. coli* bacteria produce a red color because it was included into Gram negative bacteria. And Gram staining on *S. aureus* bacteria produces a purple color because it was included into Gram positive bacteria.

*Gentamicin sulphate injection activity test in infusion preparation against Escherichia coli and Staphylococcus aureus bacteria*

After preliminary test and evaluation of the test sample, gentamicin sulphate activity was tested in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions. The results of the activity test can be seen in Table 7 and Figure 1.

The test showed a decrease in activity of gentamicin sulphate injection mixture in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions each time variation. It can be seen that the mean inhibition zone diameter of both bacteria in 5% dextrose-0.225% NaCl greater than in 0.9% solution either in 0 min to 120 min. The larger of the inhibit zone as well as its ability to inhibit bacterial growth, this shows

Table 5: Sterility testing of mixing results of gentamicin sulphate in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions.

| Time (Day) | 0.9% NaCl | | 5% dextrose-0.225% NaCl | |
|---|---|---|---|---|
| | FTM | TSB | FTM | TSB |
| 1 | - | - | - | - |
| 2 | - | - | - | - |
| 3 | - | - | - | - |
| 5 | - | - | - | - |
| 7 | - | - | - | - |
| 10 | - | - | - | - |
| 14 | - | - | - | - |

Notes: + Turbid (the growth of microorganisms)
- Not turbid (no growth of microorganisms)

Table 6: Biochemical Test of Bacteria.

| Test | *E.coli* | | *S.aureus* | |
|---|---|---|---|---|
| | R | Test | R | Test |
| Glucose | + | + | + | + |
| Sacarose | + | - | + | + |
| Manose | + | + | + | + |
| Maltose | + | + | + | + |
| Lactose | +/- | + | + | - |
| MR | + | + | + | - |
| Indol | + | - | - | - |
| VP | - | - | - | - |
| Motility | +/- | - | - | - |
| Urease | - | - | + | - |
| TSIA | + | + | + | + |
| Citrate | + | + | + | + |

Notes: R=Reference[24]

the stability of the preparation that mixing gentamicin sulphate in infusion fluids 5% dextrose-0.225% NaCl was more stable. Testing of gentamicin sulphate activity was also performed by varying the time of use of the dosage that is 0, 30, 60, 90 and 120 minutes. There was a decrease in inhibitory zone diameter every 30 minutes on both gentamicin mixtures, this occured because the preparation of gentamicin sulphate was a single dose injection preparation, in which the single dose preparation must be used immediately after the preparation was opened. So it could be concluded that the mixture of gentamicin sulfate in both infusion fluids was more effective in *Staphylococcus aureus* bacteria than *Escherichia coli*.

In addition, based on the category of antibacterial power strength, gentamicin sulphate inhibitory zone diameter of in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions was categorized as having very strong activity. Antibacterial activity was said to be weak when the inhibitory zone diameter was <5 mm, while when the inhibitory zone diameter was 5-10 mm, strong when the inhibitory zone dimension was 10-20 mm, and very strong if the inhibition zone 20-30 mm[22].

*Data analysis*

The data used first in the normality test, if the data was normally distributed then can be analyzed using ANOVA. Therefore it was necessary to test the data normality first. Data was said to be normally distributed if the value of significance was greater than the error rate (sig.> α), where α was used at 5% or 0.05. Based on ANOVA test results obtained, for bacteria *Escherichia coli* known F value calculated for both infusion fluid of 0.706 or significance value of 0.411. A greater significant value than α (<0.05) suggest that $H_0$ received means the mixture of gentamicin sulphate in intravenous fluid did not affect the activity of gentamicin sulphate. For the variation of time of use, the value of F arithmetic 4,235 or significant value of 0.012. The value of significance less than the value of α (0.012 <0.05) indicates that reject $H_0$ and receive $H_1$. So, there were effects of variation of time of use on gentamicin sulfate activity. For the third hypothesis, value of F arithmetic 0,030 or significance value of 0.998. The value of significant was greater than the value of α (0.998> 0.05) which mean accept $H_0$, that there was no effect of interaction between infusion fluid and time variation on the activity of gentamicin sulphate.

While ANOVA test results for bacteria *Staphylococcus aureus* known F value calculated for both infusion fluid amounted to 4.709 or significant value of 0.042. The significance value was smaller than the value of α (0.042 <0.05) indicating that reject $H_0$ and $H_1$, which mean there was influence of infusion fluid of in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions to activity of gentamicin sulphate. For the variation of time of use, the value of F arithmetic 7,403 or significant value of 0.001. The value of significant smaller than the value of α (0.001 <0.05) indicated that reject $H_0$ and receive $H_1$. This results an effect of variation of time of use on the activity of gentamicin sulphate. And for the third hypothesis, value of F arithmetic 0,010 or significant value of 1. This significance value was greater than the value of α (1> 0.05) which means accept $H_0$, ie there was no effect of interaction between infusion fluid and time variation on the activity of gentamicin Sulphate.

The tukey test was performed as a further test, if the ANOVA processing results rejected $H_0$. The results showed that the variation of time (0, 30, 60, 90, 120 minutes) gave insignificant influence to each other. However, in minutes 0 and 120 minutes the most significant effect, this could be seen from the significant value (0.009 <0.05) and has the largest average difference compared to other mixing time variations. Based on these results, it can be concluded that the most effective time in inhibiting bacterial growth is in the early minutes of mixing.

While in the bacterium *S. aureus* tukey test was only done on the variation of time of use, because the infusion fluid there were only 2 variations so it was definitely both were on a different subset. From the result of the tukey test it can be seen that on the variations of time (0, 30, 60, 90, 120 min) gave insignificant influence to each other. However, in minutes 0 and 120 minutes the most significant effect, this can be seen from the significant (sig <0.05) and has the largest average difference compared to other mixing time variations. Based on these results it can be concluded that the most effective time in inhibiting bacterial growth was in the early minutes of mixing. To know which

Table 7: Results of testing activity of gentamicin sulphate in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions against *Escherichia coli* and *Staphylococcus aureus* bacteria.

| Sample | Time Variations (Minutes) | Average inhibit zone diameter (cm) | |
|---|---|---|---|
| | | *E.coli* | *S.aureus* |
| Gentamicin Sulphate in 0.9% NaCl Infusion Liquid | 0 | 2,47044 | 2,71056 |
| | 30 | 2,36733 | 2,61422 |
| | 60 | 2,26133 | 2,51956 |
| | 90 | 2,20511 | 2,45011 |
| | 120 | 2,08178 | 2,37533 |
| | Gentamicin Sulphate | 2,90844 | 3,39089 |
| | 0.9% NaCl | 0 | 1,32467 |
| Gentamicin Sulphate in 5% dextrose-0.225% NaCl Infusion Liquid | 0 | 2,52111 | 2,81556 |
| | 30 | 2,40978 | 2,72 |
| | 60 | 2,35733 | 2,62311 |
| | 90 | 2,234 | 2,54044 |
| | 120 | 2,13622 | 2,45867 |
| | Gentamicin Sulphate | 3,13422 | 3,29456 |
| | 5% dextrose-0.225% NaCl | 0 | 0 |

infusion fluids have a better effect on gentamicin sulphate activity, it can be seen in Figure 2 below:

Based on Fig. 2 gentamicin sulphate mixed with intravenous fluid 5% dextrose-0.225% NaCl gave a larger inhibitory zone diameter at any time variation when compared to gentamicin sulphate mixed with 0.9% NaCl solution. Therefore gentamicin sulphate mixed in 5% dextrose-0.225% NaCl was more stable.

## CONCLUSIONS

Gentamicin sulphate in infusion of 0.9% NaCl and 5% dextrose-0.225% NaCl solutions had no effect on antibacterial activity to *E. coli*, but had an effect to *S. aureus.*

The mixing time used has an effect on the antibacterial activity of *E. coli* and *S. aureus*, both in 0.9% NaCl and 5% dextrose-0.225% NaCl solutions, where the longer mixing time gentamicin sulphate activity decreases.

The interaction between 0.9% NaCl and 5% dextrose-0.225% NaCl solutions with time of use did not affect the antibacterial activity of *E. coli* and *S. aureus.*

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