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The Effect of Mercury Exposure to Escherichia Coli Bacteria Resistant to Mercury and Escherichia Coli Esbl in Vitro

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The Effect of Mercury Exposure to *Escherichia Coli* Bacteria Resistant to Mercury and *Escherichia Coli* Esbl in Vitro

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ABSTRACT

Background: The level of pollution in Indonesia is still very high, consist of water pollution, air pollution and soil pollution. Mercury is one of the heavy metals that pollutes the waters of the sea, while *Escherichia coli* is exposed to mercury will try to defend itself by doing mercury detoxification so that it can live in an environment that contains mercury. *Escherichia coli* that tries to defend itself from mercury exposure in the environment will experience a change in its genes into mercury resistant *Escherichia coli*. In plasmids or transposons, it might also stimulate the formation of resistance genes for some antibiotics, include producing the ESBL enzyme, so that it can convert non ESBL *Escherichia coli* into ESBL *Escherichia coli*. **Objective:** This study aims to prove that the repeated exposure of mercury will change non ESBL-mercury sensitive *Escherichia coli* into ESBL- mercury resistant *Escherichia coli*. **Method:** This was an experimental study with 27 non-ESBL *Escherichia coli* isolates as identified from Phoenix. Non-ESBL *Escherichia coli* clinical isolates were tested by giving exposure to HgCl₂ with concentrations of 0.02 ppm, 0.10 ppm, 0.20 ppm for 1-14 days until mercury resistant *Escherichia coli* was formed, and then ESBL screening was tested by giving *Cefotaxime* exposure to them. **Results:** On the first day of mercury exposure, there were 9 isolates of 0.02 ppm HgCl₂ resistant *Escherichia coli*, 9 isolates of 0.10 ppm HgCl₂ resistant *Escherichia coli*, 9 isolates of 0.20 ppm HgCl₂ resistant *Escherichia coli*. Furthermore, this *Escherichia coli* isolate was exposed to *Cefotaxim* as ESBL screening. The final results of post-exposure HgCl₂ 0.02 ppm was obtained 3 (33.3%) isolates were still sensitive to *Cefotaxime* and 6 (66.7%) isolates that were resistant to *Cefotaxime*. The final results of post-exposure HgCl₂ 0.10 ppm was obtained all 9 (100%) isolates that were resistant to *Cefotaxime*. The final results of post-exposure HgCl₂ 0.20 ppm obtained 2 (22.2%) isolates were still sensitive to *Cefotaxime* and 7 (77.8%) isolate were resistant to *Cefotaxime*. **Conclusion:** *Escherichia coli* in urine had the phenotypic change into mercury resistant *Escherichia coli*. Mercury exposure of 0.02 ppm, 0.10 ppm, 0.20 ppm for 1 day in vitro on isolates of non ESBL-mercury resistant *Escherichia coli* caused changes in 22 isolates of *Escherichia coli* in urine.

Introduction

The level of pollution in Indonesia is still very high in the form of water, air, and soil pollution. This pollution will be a serious threat to the Indonesian state with all its potential. Humans can be exposed to

mercury not only from environmental pollution but can also be caused by the use of cosmetic ingredients containing mercury, consuming food/drinks containing mercury, or due to direct contact with materials containing mercury (Saraswati and Fahrudin, 2012)

One of these wastes is that heavy metals can be sourced from factories or industries. The toxic and dangerous properties of heavy metals are shown by the physical and chemical properties of the material, both in terms of quality and quantity. Mercury is a heavy metal that pollutes marine waters, caused by natural factors and human activities that dispose of its waste into the waters. Sources of mercury waste can come from the cosmetics industry, electronics, paint manufacturing industry, denture manufacturing, gold smelting, as a catalyst, and others (Palar, 2008).

Several researchers have researched the presence of antibiotic-resistant bacteria coexisting in mercury-resistant bacteria. One of the studies that have been carried out in the research that has been conducted by Ririn (2013) from the Biology Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar. In her research, the isolation of bacteria from seawater samples from Losari Beach was carried out using Nutrient Media for Synthetic Sea Water Agar (NA ALS) added with HgCl with a concentration level of 0.0002 mg/ml (0.02µg / ml). Then the bacterial isolates were tested for antibiotic resistance using antibiotic paper disks (Oxoid) including Ampicillin 10µg, Novobiocyn 30µg, Chloramphenicol 30µg, Cefoperazone 75µg, and Ofloxacin 5µg. Three types of isolates are mercury (Hg) resistant and the antibiotics obtained are: mercury I resistant bacteria isolates which are also resistant to Ofloxacin 5µg, mercury II resistant bacteria isolates that are resistant to Ampicillin 10µg and mercury III resistant bacteria isolate that is resistant to all types of antibiotics (Ririn, 2013).

Bacteria exposed to mercury will try to defend themselves by detoxifying mercury, so that these bacteria can live in

environments that contain mercury. Bacteria that try to defend themselves from exposure to mercury in the environment will experience a change in their genes to become mercury-resistant bacteria. Mercury detoxification by mercury-resistant bacteria occurs because bacteria have the *mer operon* gene (Boyd, 2012).

The structure of the operon *mer* is different for each type of bacteria. Generally, the operon *mer* structure consists of the metalloregulator gene (*merR*), the mercury transfer gene (*merT*, *merP*, *merC*), the mercury reductase gene (*merA*), and the organo mercury lyase (*merB*). Bacteria that only have the mercury reductase (*merA*) gene are called narrow spectrum mercury-resistant bacteria. Several bacteria have in addition to the *merA* gene, also the *merB* gene, so these bacteria are called broad-spectrum mercury resistant bacteria. The protein *merA* gene is responsible for the presence of the enzyme mercury reductase. The mercury reductase enzyme will give 2 electrons to NADPH or NADP so Hg²⁺ turns into Hg⁰ which is volatile and will be removed from the cell, while the *MerB* protein has the function of catalyzing the breaking of the mercury-carbon bond so that organic compounds and Hg²⁺ ions are produced. (Barkay et al., 2003).

In plasmids or transposons, it may also stimulate the formation of genes coding for resistance to several antibiotics, one of which is the enzyme-producing bacteria ESBL, where ESBL is mostly derived from the TEM and SHV enzymes, and in lesser amounts than CTX (Walker, 2015) where the ESBL coding gene can be either chromosomal or plasmidal. Changes in *E. coli* were initially sensitive to mercury without ESBL, but because they live in an environment that has high mercury levels, it can change to ESBL mercury-resistant *E.*

coli. This is what makes researchers feel the need to do this research because with a large amount of mercury that is in nature, both in the environment, including air, soil, water, besides that there are animals that have been contaminated with mercury that we consume, many of the items we use are used. contains mercury ranging from cosmetics, medical equipment, factories, etc. and if we are exposed to these items then we will be contaminated with the mercury material and then the mercury levels in our bodies will be high. If there are bacteria that enter our bodies, then there is a possibility that bacteria are initially sensitive to non-ESBL mercury will become resistant to ESBL mercury, which is very dangerous for us and complicates the treatment.

In this study, the researchers researched bacteria that were resistant to mercury (Hg) and ESBL whose isolates were taken from urine specimens at the Clinical Microbiology Unit of Dr. Soetomo Hospital, Surabaya.

Methods

This research is an experimental study, by providing treatment and observation of the results of urine culture that grows mercury susceptible non-ESBL *E. coli* bacteria. The *E. coli* strain is a clinical urine isolate stored in the Clinical Microbiology

Unit of the Dr. Soetomo Hospital, Surabaya.

The study population was clinical isolates of *E. coli* stored from urine specimens in the Clinical Microbiology Unit of Dr. Soetomo Hospital, Surabaya. The sample of this study was clinical isolate *E. coli* non ESBL stored from urine specimens in the Clinical Microbiology Unit of Dr. Soetomo Hospital, Surabaya. ppm to non-ESBL *E. coli* bacteria for 1-14 days.

The number of samples needed in this study is:

$$(r-1)(t-1) \geq 15$$

$$(r-1)(3-1) \geq 15$$

$$(r-1)(2) \geq 15$$

$$(r-1) \geq 3$$

$$r \sim 9$$

Information :

r = total samples
t = total treatment

So this study used 9 isolates of *E. coli* susceptible mercury non ESBL.

The Sample's Criteria

Inclusion criteria: *E. coli* comes from urine specimens in the Clinical Microbiology Unit of Dr. Soetomo Hospital and the *E. coli* isolate was not ESBL *E. coli* based on the identification and sensitivity testing with Phoenix or Vitex 2. Exclusion

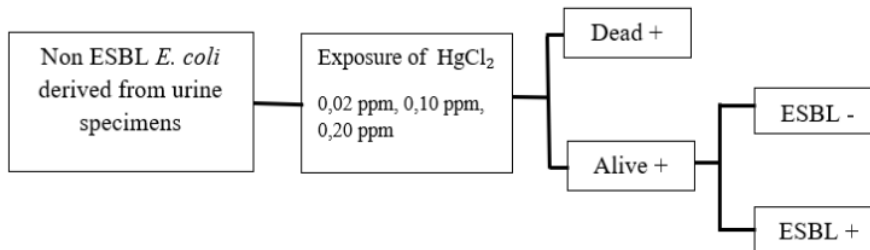


Figure 1. Research Diagram

criteria: stored *E. coli* isolates that do not grow.

Sampling Technique

A sampling of stored clinical *E. coli* isolates from urine specimens in the Clinical Microbiology Unit, was carried out using consecutive sampling techniques. Each sample that meets the research criteria is taken for the required sample size.

Research Variables And Operational Definitions

Research Variables

1. Independent Variables: Repeated exposure of HgCl₂ in 0,02 ppm, 0,10 ppm, and 0,20 ppm for 1 - 14 days
2. Dependent Variables: Occurrence of *E. coli* resistance Mercury and which categorized as ESBL produce

The research was conducted at the Clinical Microbiology Unit of Dr. Soetomo Hospital, Surabaya. From May 2019 - August 2019. The main material used in this study was clinical isolates of *E. coli* bacteria non ESBL stored from urine specimens in the Clinical Microbiology Unit Dr. Soetomo Surabaya. Additional materials used in this study were Mueller-Hinton agar, HgCl₂ liquid with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm, 30µg *Cefotaxime* antibiotic disk.

Difusion Method

The Techniques That Used In Laboratory

1. The media was used is MHA (Mueller-Hilton Agar)
2. Empty disc which dropped by HgCl₂ liquid with 0,02 ppm, 0,10 ppm, and 0,20 ppm concentration

The Procedure:

1. Do the streaking of the bacteria that will be tested on all surfaces so that they are evenly distributed.
2. Place an empty disc dripped with liquid HgCl with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm on the agar surface.
3. Plate 9-10 cm in diameter.
4. Furthermore, the plates were incubated at 35 ° C for 24 hours.
5. The zone diameter is measured in mm.

The Interpretation Result Of Treated *E. coli*

1. Susceptible (S)

The tested isolate can be inhibited with this antimicrobial at the recommended dosage. It is said to be susceptible if an inhibition zone is obtained.

2. Resistance (R)

This means that isolates cannot be inhibited by these antimicrobials. It is said to be resistant if an inhibition zone is not found.

Research Work Procedure

Mercury Disc Preparation

- a. HgCl₂ disc 0,02 ppm concentration is a mercury which diluted with Cl₂.
- b. HgCl₂ disc 0,10 ppm concentration is a mercury which diluted with Cl₂.
- c. HgCl₂ disc 0,20 ppm concentration is a mercury which diluted with Cl₂.

Bacteria Isolate Preparation

1. *E. coli* isolates stored from urine specimens in the Clinical Microbiology Unit have been identified and tested for antibiotic sensitivity.
2. Isolates that met the criteria as *E. coli* bacteria and the degree of sensitivity to *Cefotaxime*, *Ceftazidime*, *Ceftriaxone*, and *Aztreonam* (Phoenix-BD, CLSI M100-S 2019) were selected 3 - 5 colonies from MacConkey agar media using cotton stick swabs.

3. Then it is suspended in a 0.9% NaCl solution in a glass tube until it reaches a germ density of 0.5 McFarland which is equivalent to 1.5×10^8 CFU / mL.

Hg Disc and Cefotaxime Preparation

1. The 0.5 McFarland suspension was densely planted onto the MHA surface. Placed the disc which already dripped by HgCl₂ with 0,02 ppm, 0,10 ppm, and 0,20 ppm concentration in the surface of MHA.
2. MHA plates were incubated for 18-24 hours at $35^\circ\text{C} \pm 2^\circ\text{C}$.

Isolate Preparation for 2 Days of Exposure and Later

1. Colonies that are on the edge of the inhibition zone are taken with a sterile cotton swab
2. Then it was suspended in a 0.9% NaCl solution to reach 0.5 McFarland turbidity.
3. The 0.5 McFarland suspension was densely planted onto the MHA surface. On the surface of the MHA, a disc that has been dripped with HgCl is placed with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm
4. MHA plates were incubated for 18-24 hours at $35^\circ\text{C} \pm 2^\circ\text{C}$.

Read The Sensitivity Test of Gentamycin and CTX

1. The sensitivity test to HgCl₂ is said to be susceptible if the diameter of the zone of inhibition is $\geq 15\text{mm}$, and resistant if $\leq 12\text{mm}$.
2. The sensitivity test to Cefotaxime is said to be susceptible if the zone of inhibition is $\geq 26\text{mm}$ and resistant if the zone of inhibition is $\leq 22\text{mm}$
3. The ESBL screening test is said to be positive if it is resistant to Cefotaxime

Research Data Collection Procedures

The research data collection procedure consisted of observing and recording the results of the observations into the table that had been provided.

Data Processing and Analysis Techniques

Processing techniques and data analysis were obtained statistically using Chi-square analysis.

Result

The samples of this study were 27 *Escherichia coli* isolates obtained from clinical specimens taken by consecutive sampling. Samples were collected from May 2019 to August 2019. *E. coli* isolates obtained from the phoenix automatic machine were retested using the Kirby-Bauer method, the result are in table 5.01. The re-sensitivity test using the Kirby-Bauer antibiotic disc diffusion method was carried out to equate the methods used during the screening test and ESBL confirmation.

All isolates that met the inclusion-exclusion criteria were exposed to HgCl₂ at concentrations of 0.02 ppm, 0.10 ppm, 0.20 ppm, and Cefotaxime (CTX). The exposure was carried out every day in a petri dish containing Mueller Hinton Agar using HgCl₂ with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm with a maximum length of research for 1 day. If within 1 day of the study a positive result of mercury resistance is obtained, then it will be continued by providing exposure to screen for ESBL.

The research was carried out using the Kirby-Bauer method, by dripping a blank disc with drops of HgCl₂ with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm then a disc containing HgCl₂ with a concentration of 0.02 ppm, 0.10 ppm, 0.20

ppm was exposed on the surface of the MH agar plate. After being incubated for 24 hours, the results are in the following table. The basis for determining the concentration of mercury used in this study is based on research conducted by Natalia from Unair, which resulted in a mercury concentration of 1.22 ppm. *E. coli* bacteria were still alive. So the researchers used mercury with a concentration of 0.02 ppm, 0.10 ppm, and 0.20 ppm in the hope that researchers would get results whether in an environment containing mercury with a concentration of 0.02 ppm *E. coli* bacteria could still survive. This research technique uses the Kirby-Bauer method by dripping a blank disc with a concentration of 0.02 ppm of HgCl₂, 0.10 ppm, 0.20 ppm then a disk containing HgCl₂ with a concentration of 0.02 ppm, 0.10 ppm, 0, 20 ppm was exposed on the surface of the MH agar plate.

The method used is based on the direction of Edy Bagus who is a professor in Microbiology, Airlangga University, which is the result of his experience and research in Japan.

Table 1. *E. coli* Resistance To HgCl₂ Exposure With Concentration Of 0,02 Ppm (n=9), 0,10 ppm (n=9), 0,20 ppm (n=9) by kirby-bauer method (total n=27)

No	Exposure	Exposure Day - 1	
		Sensitivity	Resistance
1	HgCl ₂ 0,02 ppm	0 (0%)	9 (100%)
2	HgCl ₂ 0,10 ppm	0 (0%)	9 (100%)
3	HgCl ₂ 0,20 ppm	0 (0%)	9 (100%)

On the first day of exposure to HgCl₂ using the Kirby-Bauer method using a blank disk that has been dripped with HgCl₂ with a concentration of 0.02 ppm, 9 (100%) resistant isolates were obtained, by exposure to HgCl₂ with a concentration of 0.10 ppm obtained 9 (100 %) isolates that were resistant and by exposure to HgCl₂

with a concentration of 0.20 ppm, obtained 9 (100%) resistant isolates.

The next stage of the research was carried out using the results of the first treatment, by exposure to HgCl₂ concentrations of 0.02 ppm, 0.10 ppm, 0.20 ppm then exposed to the *Cefotaxime* disk as a screening for ESBL.

Table 2. *E. coli* Resistance to *Cefotaxime* post HgCl₂ Exposure in 0,02 ppm (n=9), 0,10 ppm(n=9), 0,20 ppm (n=9) Concentration with Kirby-Bauer Method (total =27)

No	Exposure	Exposure Day - 1 CTX	
		Sensitivity	Resistance
1	HgCl ₂ 0,02 ppm	3 (33,3%)	6 (66,7%)
2	HgCl ₂ 0,10 ppm	0 (0%)	9 (100%)
3	HgCl ₂ 0,20 ppm	2 (22,2%)	7 (77,8%)

On the first day of exposure to *Cefotaxime* on the results of 0.02 ppm HgCl₂ exposure, 3 (33.3%) isolates were still susceptible to *Cefotaxime* and 6 (66.7%) isolates were resistant to *Cefotaxime*. On the first day of exposure to *Cefotaxime* on the results of exposure to HgCl₂ 0.10 ppm, there were no (0%) isolates that were still susceptible to *Cefotaxime* and 9 (100%) isolates that were resistant to *Cefotaxime*. On the first day of exposure to *Cefotaxime* on the results of exposure to HgCl₂ 0.20 ppm, 2 (22.2%) were still susceptible to *Cefotaxime* and 7 (77.8%) isolates were resistant to *Cefotaxime*.

All isolates from *E. coli* became resistant after exposure to HgCl₂ with concentrations of 0.02 ppm, 0.10 ppm, 0.20 ppm on the first day of exposure. There was no difference between exposure to HgCl₂ with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm. In *Cefotaxime* exposure to the emergence of ESBL in *Escherichia coli* from urine specimens, different results were obtained at each HgCl₂ concentration previously exposed.

Discussion

In this study, 27 isolates were exposed to HgCl₂ with a concentration of 0.02 ppm (9 isolates), 0.10 ppm (9 isolates), 0.20 ppm (9 isolates), it was found that all *E. coli* isolates from urine were resistant *E. coli* mercury. This is following research conducted by Hinonaung (2013) from the Chemistry Section of the Faculty of Medicine, Sam Ratulangi University. The research was carried out in several stages, mentioned the isolation stage of mercury resistant bacteria which aims to isolate the bacteria present in tartar, urine, and fecal samples. The results obtained from 6 isolates found four types of mercury-resistant bacteria in tartar sediment, feces, urine, there are *Enterococcus*, *Bacillus sp*, *Streptococcus sp*, *E. coli*. The results of the resistance test to mercury showed that 3 bacteria can survive up to a 40 ppm HgCl₂ concentration, namely *Enterococcus*, *Bacillus sp*, and *Streptococcus sp*. At a concentration of 20 ppm of Phenyl Mercury, 2 bacteria can survive, namely *Bacillus sp*, *E.coli* (Hinonaung, 2013).

In this study, HgCl₂ was used with a small concentration of 0.02 ppm to prove that with a very small concentration, *E. coli* had adapted and became mercury resistant. Heavy metal contamination, including mercury in humans, will continue to increase in line with the increasing exploitation of various natural sources where heavy metals are contained. This is related to the properties of heavy metals:

1. Difficult to degrade, so that problems accumulate in the aquatic environment and its natural presence is difficult to decompose.
2. Can accumulate in organisms and will endanger the health of humans who consume these organisms.

3. Easily accumulates in sediments, so that the concentration is always higher than the metal concentration in water. Besides, sediment is easily suspended by the movement of water masses which will dissolve the metal it contains back into the water so that the sediment becomes a potential source of pollution in a certain time scale.

Humans are exposed to heavy metals, such as mercury because the properties of heavy metals above will cause them to have mercury levels in their bodies (Widowati et al., 2008). So that the bacteria that are in the human body will be in an environment that contains mercury. Bacteria that live in environments exposed to mercury will try to defend themselves by detoxifying mercury so that these bacteria can live in environments that contain mercury. Bacteria that try to defend themselves from exposure to mercury in the environment will experience a change in their genes to become mercury-resistant bacteria.

Mercury detoxification by mercury-resistant bacteria occurs because bacteria have the *mer operon* gene. The structure of the *operon mer* is different for each type of bacteria. Generally, the operon mer structure consists of the metalloregulator gene (*merR*), the mercury transfer gene (*merT*, *merP*, *merC*), the mercury reductase gene (*merA*), and the organo mercury lyase (*merB*). Protein The *merA* gene is the gene responsible for the presence of the enzyme mercury reductase. The mercury reductase enzyme will give 2 electrons to NADPH or NADP so that Hg²⁺ turns into Hg⁰ which is volatile and will be removed from the cell (Brown et al, 2002), while the *MerB* protein has the function of catalyzing the breaking of the mercury-carbon bond resulting in organic compounds and Hg²⁺ + ions. (Barkay et al., 2003).

However, there is another possibility besides the allegations above is that *E. coli* that enters the human body is already an *E. coli* bacteria that are already resistant to mercury. This requires further research to prove the truth of the allegations above regarding the presence of mercury-resistant *E. coli* bacteria found in the body of this research sample.

The results of the first stage were *E. coli* bacteria that had been exposed to HgCl₂ with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm which turned out to be all mercury resistant, then the mercury-resistant *E. coli* bacteria were exposed to *Cefotaxime* discs for ESBL screening. Previously, *Cefotaxime* was exposed to *E. coli* on the primary plate and the results were all are *Cefotaxime* susceptible *E. coli*. After that, the *E. coli* from the primary plate was exposed to mercury with a concentration of 0.02 ppm, 0.10 ppm, 0.20 ppm, then the results of the previous exposure were exposed to *Cefotaxime* discs for ESBL screening. The results of changes in the phenotypic properties of several non-ESBL *E. coli* isolates became ESBL after being exposed to *Cefotaxime* on the first day.

On the first day of exposure to *Cefotaxime* on the results of 0.02 ppm HgCl₂ exposure, 3 (33.3%) isolates were still susceptible to *Cefotaxime* and 6 (66.7%) isolates were resistant to *Cefotaxime*. On the first day of exposure to *Cefotaxim* on the results of exposure to HgCl₂ 0.10 ppm, there were no (0%) isolates that were still susceptible to *Cefotaxime* and 9 (100%) isolates that were resistant to *Cefotaxime*. On the first day of exposure to *Cefotaxime* on the results of exposure to HgCl₂ 0.20 ppm, 2 (22.2%) were still susceptible to *Cefotaxime* and 7 (77.8%) isolates were resistant to *Cefotaxime*.

This is consistent with research conducted by Ayu (2013) from the Biology Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar. In her research, the isolation of bacteria from seawater samples from Losari Beach was carried out using nutrient agar for Synthetic Seawater (NA ALS) media with added mercury at a concentration level of 0.02 µg / ml. Then the bacterial isolates were tested for antibiotic resistance using antibiotic paper disks (Oxoid) including Ampicillin 10 µg, Novobiocyn 30 µg, Chloramphenicol 30 µg, Cefoperazone 75 µg, and Ofloxacin 5 µg. Three types of isolates are resistant to mercury (Hg) and the antibiotics obtained are: mercury I resistant bacteria isolates which are also resistant to Ofloxacin 5 µg, mercury resistant bacteria II isolates that are resistant to Ampicillin 10 µg and mercury III resistant bacteria isolate that is resistant to all types of antibiotics.

In plasmids or transposons, it may also stimulate the formation of genes coding for resistance to several antibiotics, one of which is in the enzyme-producing bacteria ESBL, where ESBL is mostly derived from TEM and SHV enzymes, and in lesser amounts than CTX (Walker et al., 2015) where ESBL coding genes can be chromosomal or plasmidal. The resistance mechanism of each class of antibiotics can be different from other groups. Some researchers mention cross-resistance between different antibiotic classes (Canton and Coque, 2006; Talan et al., 2016). Changes in *E. coli* which are initially sensitive to non-ESBL mercury, but because living in an environment that has high mercury levels can changes it to ESBL mercury-resistant *E. coli* (Chojnacka, 2010)

Conclusion

The *E. coli* bacteria in the urine in sample body is mercury-resistant *E. coli*. This is proved by the results of exposure to mercury with concentrations of 0.02 ppm, 0.10 ppm, 0.20 ppm. It was found that all 30 samples of the study were mercury-resistant *E. coli*. Exposure to *Cefotaxime* for 1 day by invitro to mercury-resistant *E. coli* isolates non ESBL caused changes in 22 *E. coli* isolates in the urine. This proves that exposure to HgCl₂ with various concentrations to *E. coli* isolates can accelerate the phenotic change from non-ESBL *E. coli* to ESBL *E. coli*.

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