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THE SCREENING OF POTENTIAL ANTIBIOTICS FROM HOSPITAL WASTEWATER IN TROPICAL REGION (CASE STUDY AT PALEMBANG, SOUTH SUMATRA, INDONESIA)

I. KURNIAWAN, S. NASIR, HERMANSYAH AND MARDIYANTO

Abstract

Clean water is a primary one of the causes of water quality degradation is pollution through the residue of human activity. Hospital is a potential pollutant source with the characteristics of hazardous and toxic wastewater. The components of pollutants in the wastewater of hospital is still limited investigated is antibiotic. The regulation of hospital wastewater in Indonesia as tropical and developing countries do not regulate antibiotics, while in the world the issue of zero tolerance of antibiotics becomes an important concern. Antibiotics have a persistent and resistant impact for the environment. In this research, screening for antibiotics by using samples from one type B hospital in Palembang City, Indonesia is done. Hospital wastewater samples in the non permanent pond are the residue of all process activities mixed in one pond. Screening using High-Resolution Mass Spectrometry (LC / Q-TOF / MS) 6500 Series for the Detection of Pharmaceuticals in Water is used to detect the antibiotics. The result of laboratory analysis shows that there are five types of antibiotics detected, namely Ciprofloxacin, Lincomycin, Metronidazole, Netilmicin, and Ofloxacin/Levofloxacin. The qualitative analysis concluded that Ciprofloxacin is one of type potential antibiotics in the hospital wastewater. The result of this research recommends that the Indonesian Government should revise the regulation about antibiotics in the hospital wastewater.

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THE SCREENING OF POTENTIAL ANTIBIOTICS FROM HOSPITAL WASTEWATER IN TROPICAL REGION (CASE STUDY AT PALEMBANG, SOUTH SUMATRA, INDONESIA)

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ABSTRACT

Clean water is a primary one of the causes of water quality degradation is pollution through the residue of human activity. Hospital is a potential pollutant source with the characteristics of hazardous and toxic wastewater. The components of pollutants in the wastewater of hospital is still limited investigated is antibiotic. The regulation of hospital wastewater in Indonesia as tropical and developing countries do not regulate antibiotics, while in the world the issue of zero tolerance of antibiotics becomes an important concern. Antibiotics have a persistent and resistant impact for the environment. In this research, screening for antibiotics by using samples from one type B hospital in Palembang City, Indonesia is done. Hospital wastewater samples in the non permanent pond are the residue of all process activities mixed in one pond. Screening using High-Resolution Mass Spectrometry (LC / Q-TOF / MS) 6500 Series for the Detection of Pharmaceuticals in Water is used to detect the antibiotics. The result of laboratory analysis shows that there are five types of antibiotics detected, namely Ciprofloxacin, Lincomycin, Metronidazole, Netilmicin, and Ofloxacin/Levofloxacin. The qualitative analysis concluded that Ciprofloxacin is one of a type potential antibiotics in the hospital wastewater. The result of this research recommends that the Indonesian Government should revise the regulation about antibiotics in the hospital wastewater.

KEY WORDS : Clean water, Environment, Antibiotics, Hospital wastewater, Ciprofloxacin

INTRODUCTION

World Health Statistics reported by WHO in 2015 shows that about 85% of the Indonesian population needs access to clean water for consumption. The community in the late decades concerns the importance to solve the environmental problems, especially about clean water conservation. International Water Institute predicts in 2025 Java and the others Islands in Indonesia will have a crisis of clean water. The water global scarcity has increased and is needed the other sources to make it balance with the water need because existing

water sources has not been used because of the pollution and wastewater. The problem of clean water sources because of containing components of polluters in high concentrations, such as heavy metals, toxic compounds, and microbial pathogens.

The data released by WWF world organization in its report explain that the data between 1930 to 2000 about the production of chemicals caused by human activities increased from 1 million to 400 million/year. The statistical data published by Euro Stat in 2013 explain that between 2002 and 2011 more than 50% of the total chemical production produced the dangerous compounds and 70% out of them has a

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significant impact on the environment. Besides, human activities have affected the contamination of water resources with micropollutants biological, such as viruses and bacteria that are toxic (Xie, 2012; Periasamy and Sundaram, 2013; Sui *et al.*, 2015).

Contaminants transportation in the aquatic environment is described in Figure 1 (the model source-line-receptors) describes the path of pollution of wastewater from various sources, including hospitals, which can be harmful to the environment especially for human health and natural ecosystems. The residual chemicals which go into the exhaust system will go towards the body of water, such as river, lake or sea. On the low speed, this waste flows into the groundwater if there is a highly enough concentration or if there are gaps and cracks on the ground surface. That fact is urgent and becomes a challenge in doing particular observation and investigation which must be done to anticipate micro pollutants contaminants entering the body of water through various research and studies (Jiang *et al.*, 2013).

Hospital is one of the pollutant sources which results from wastewater from drugs. The installation

of wastewater treatment owned by hospital generally is not be able to eliminate these compounds (Verlicchi *et al.*, 2012). The residue of the chemical and pharmaceuticals from hospital wastewater can not be eliminated by the installation system and generally, it becomes residue, virus, and bacteria and then transforms as a multiresistant agent in the environment (Yamina *et al.*, 2014; Yuan *et al.*, 2015). Several studies which have been done (Zorita *et al.*, 2009; Kosma *et al.*, 2010; Behera *et al.*, 2011; Deegan *et al.*, 2011; Jelic *et al.*, 2011; Gracia-Lor *et al.*, 2012) conclude that wastewater treatment installation owned by the hospital does not have the capability to reduce wastewater containing drugs.

The activities done in the hospital will produce the residue of drugs, one of them is antibiotics. Antibiotic is an important part of treatment because it helps to treat the humans' infection (Hirsch *et al.*, 1999). Wastewater from the health services, for example, from hospital containing chemicals and drugs which are carcinogenic and genotoxic which cause cancer and genetic abnormalities (Escher *et al.*, 2010; Orias and Perrodin, 2013; Bayer *et al.*, 2014; Sharma *et al.*, 2015).

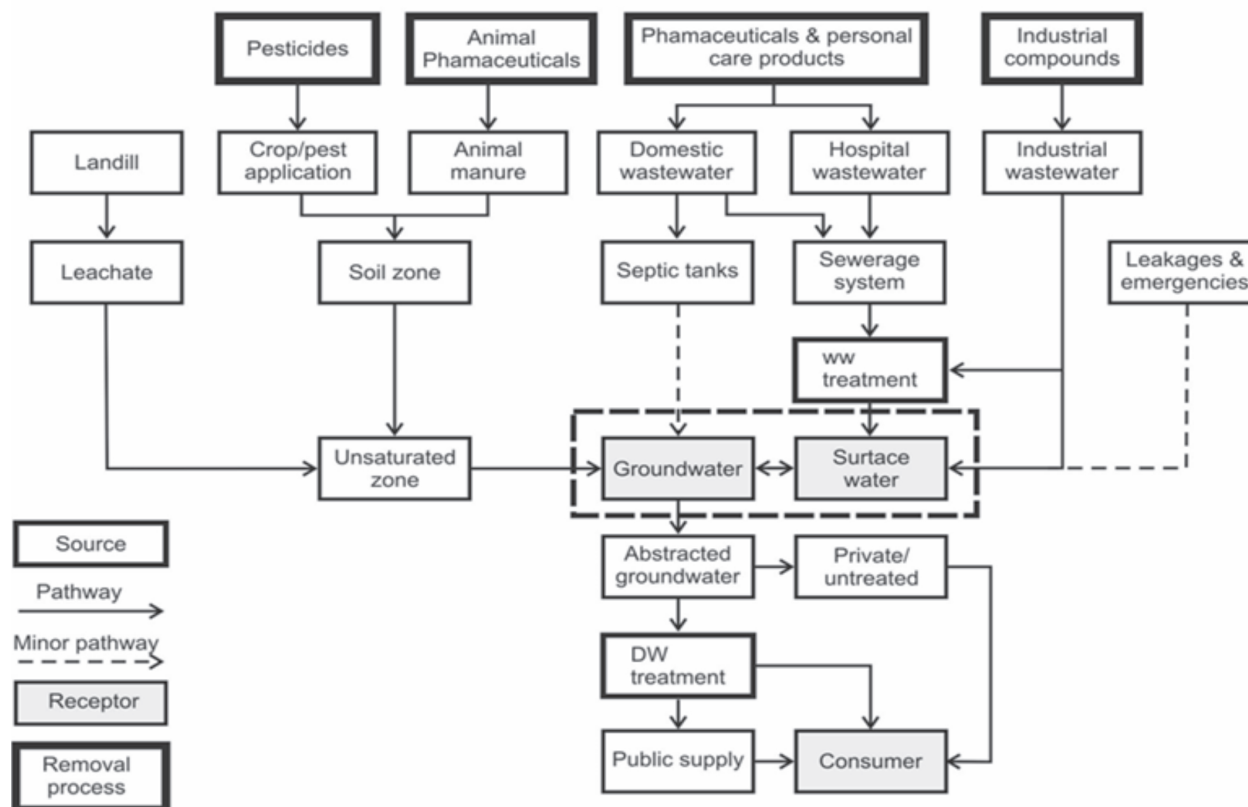


Fig. 1. Pathways of Pollution in Water (Stuart *et al.*, 2012)

Yoon *et al.* (2010) found that 31 micropollutants (Atorvastatin, Octylphenol, Gemfibrozil, Triclosan, Fluoxetine, Musk ketone, Estradiol, Progesterone, Ibuprofen, Ethynylestradiol, BHA, Testosterone, Bisphenol A, Naproxen, Benzophenone, Estrone, the TCPP, Diazepam, Dilantin, Carbamazepine, Atrazine, DEET, Trimethoprim, Sulfa-methoxazole, TCEP, Primidone, Diclofenac, Meprobamate, Caffeine, Iopromide) which are compound of the endocrine system disorder and drugs in the surface water and in the river in Seoul, South Korea in the summer and winter condition. Hamjinda *et al.* (2015) also observe the character of wastewater hospital using antibiotic compound as the research subject in the seven hospitals in Bangkok city and found some of antibiotics compound (Levofloxacin, Ofloxacin, Quinolone, Ciprofloxacin, Norfloxacin, Sulfametoksazol, Norfloxacin) which have an effect on the toxicity on several types of microorganisms (*Chlorella vulgaris* and *Scenedesmus quadricauda*), and Microcrustacean (*Moina macrocopa*).

Prayitno *et al.* (2012) conduct a research about the quality of the hospital wastewater in Malang city and some of the result of the studies about wastewater treatment quality in several hospitals in Indonesia (RSUD Kelet Jepara, RSUD Dr. Muhammad Zein Painan Sumbar, RS Permadi, RSU Prov. NTB, RSUP Dr. Wahidin Sudirohusodo Sulsel, RS PKT Bontang, RSUP Persahabatan) conclude that facts and data are relatively similar (in aspect Physics, Biology, Chemistry, and Radioactivity) and they do not focus on researching the content of drugs in the wastewater because of the limitations of technology and the regulation done by Indonesia Government does not regulate specifically about the contaminant material, whereas in the world, zero tolerance of antibiotics effect is being socialized.

The detection of micropollutants content in wastewater with specific containing drugs can be done using High-Performance Liquid Chromatography (HPLC) (Ren *et al.*, 2008; Dolar *et al.*, 2013; Mendoza *et al.*, 2015; Sabry *et al.*, 2015). According to Ferrer and Thurman (2012), the use of High-Resolution Mass Spectrometry (LC/Q-TOF/MS) with the principle of mass spectrometer enables the identification and characterization of more than 100 drugs and metabolite in the water source at small concentrations in a part per trillion (ppt). The examination method can provide a good sensitivity and selectivity in identifying the types of antibiotics which are unknown its group (untargeted) in low

concentrations of the water sample.

In this research, screening and identification of the type of antibiotics in the hospital wastewater has been done in Indonesia. The identification of antibiotic which is detected can be used as the recommendations to the Government of Indonesia. The recommendation explains about the new element polluters in the hospital wastewater which needs special regulation to keep the environmental sustainability.

MATERIALS AND METHODS

This research was conducted during August – December 2016 by taking wastewater hospital in one of the hospital type B in Palembang City, South Sumatra Province, Indonesia. Wastewater sample used is a mixed of all activities done in the hospital which has liquid characteristic and hazardous and toxic material (B3). The assumption which can be used for the same activities having the similarities in terms of the characteristic properties of the wastewater produced. A sample of the waste used is the wastewater hospital in reception pond before entering to the installation process of wastewater treatment with size 2.4 m x 1.9 m x 4 m and volume $\pm 18,2 \text{ m}^3$. The selection of the sample research was done by using purposive sampling with the criteria the hospital which has technically wastewater treatment pond located in the underground so that the confounding variable, namely rainfall and extreme weather can be neglected.

The sampling instrumentation used vandorn sampler which can be used to a specific depth. Wastewater retrieval methods used grab method and composite. Wastewater sample mixed is taken from different points (basic, middle, and the surface of the pond) in different time span, with the same volume of 2 liters and homogenized. The sample is stored in the sample bottle of polyethylene as much as 2 liters and then sent it to the examination laboratory, Anglerbiochem Laboratory Surabaya, Indonesia.

The screening process will be done to predict qualitatively the types of antibiotics contained in the wastewater by using High-Resolution Mass Spectrometry (LC/Q-TOF/MS) 6500 Series for the Detection of Pharmaceuticals in Water.

Standard operating procedure using High-Resolution Mass Spectrophotometry (LC/Q-TOF/MS) 6500 Series to examine the hospital wastewater are as follows the analysis uses the Mobile Phase

and C18 column A (10% Methanol with 5 mM Ammonium acetate), Mobile Phase B (10% Methanol with 5 mM Ammonium acetate). The wastewater sample is prepared then done the evaporation at a temperature of 40°C until dry (residue), residue then is added to methanol solvent 50%. The process uses Vortec and Ultrasonic, Centrifuge 10,000 rpm for 5 minutes, Syringe with filter 0.22 µm, and injected to a High-Resolution Mass Spectrometry (LC/Q-TOF/MS) 6500 Series.

The use of the Agilent 6540 Q-TOF/LC/MS is an instrumentation system which is integrated with some supporting software, namely Molecular Feature Extractor, Molecular Formula Generator, and Accurate Mass Databases to detect, identify, and predict the concentration of antibiotics that exist in the water.

RESULTS AND DISCUSSION

The Qualitative Antibiotics Data Consumption in Hospital

The wastewater treatment system in Hospital X uses a combination of technology systems treatment processes in physics, chemistry and biology integratedly. The pool is made of cast concrete material and means residence time - approximately 30 minutes with a processing capacity of 1000 m³/day. The wastewater treatment process is not designed to reduce the level of medicines, especially antibiotics. Antibiotics is a pollutant compound that has characteristics and requires further processing (Aamand *et al.*, 2014).

Each hospital provides services including inpatient, emergency, operations, radiology, laboratory, pharmacy, kitchen, laundry, administrative, education and training, and other supporting services. The potential discovery of residual antibiotics use that comes with wastewater from the process of hospital services is by performing sampling on the primary pond.

The activities of the Hospital X, known as a type B-Hospital, which are mainly related to medical activities will be using the medicines - either antibiotics or other medicines. Primary data collection technique of antibiotics use applies to time series method, that is a data-retrieval of antibiotic use in a period of a month or a certain period (July – September 2016), so that this data can be regarded as historical data or time series. The results of qualitative data of antibiotics consumption in

Hospital X are presented in Table 1.

Based on Table 1, the use of antibiotics in Hospital X can be classified based on the mechanism of action of the usefulness of these antibiotics, such as groups of Beta-lactam, Cephalosporins, Lincosamide, Nitroimidazole aim to inhibit the synthesis or to damage the cell wall of bacteria or protozoa. Groups of Aminoglycosides, Thiamphenicol, Tetracycline, Macrolide, Clindamycin, aim to modify or to inhibit protein synthesis. Group of Sulfonamides aims to inhibit essential enzymes in the metabolism of folate. Group of Quinolones affects the synthesis or nucleic acid metabolism.

Molecular Feature Extractor (MFE)

Molecular Feature Extractor (MFE) produces the first output from the results of wastewater samples in the form of the chromatogram of the antibiotics compound (Fig. 2, Fig. 3, Fig. 4, Fig. 5 and Fig. 6).

Molecular Formula Generator (MFG)

Molecular Formula Generator (MFG) will identify the form of the *chromatogram* in order to be matched with a particular compound formula to produce the spectrum of antibiotics (Fig. 7 Cyprofloxacin, Fig. 8 Lincomycin, Fig. 9 Metronidazole, Fig. 10 Netilmicin, and Fig. 11 Ofloxacin/Levofloxacin).

Accurate Mass Database (AMD)

The final step of the instrumentation system is Accurate Mass Database. In this step, the spectrum from MFG will be matched with the mass of molecular compounds and retention time in the library tools, then detected antibiotics types will be obtained. Five Antibiotics are detected from Hospital Wastewater in the tropical country (Table 2).

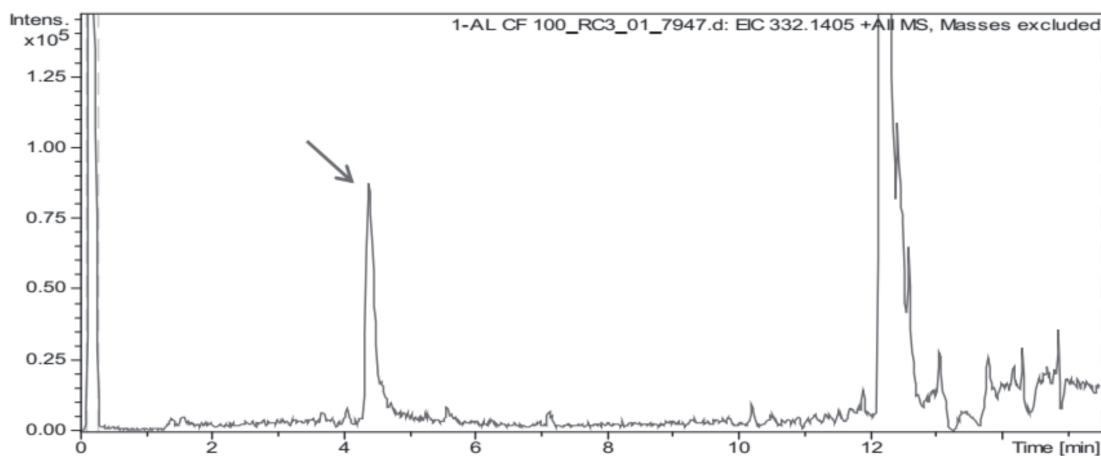
Ciprofloxacin Issue

The identification result of antibiotics type detected in waste water samples of the hospital produces 5 types of antibiotics detected from 126 types of antibiotics in which screening are conducted. The use of five types of antibiotics to patients through oral (tablets, capsules, and syrups), injection and infuse will remain the rest of antibiotics due to the improper use and disposal, hence these remains are mixed with wastewater of the hospital.

The research conducted on wastewater effluent of the hospital found that even though the wastewater treatment process had been done, but the content of

Table 1. Qualitative Data of Antibiotics Consumption in Hospital X

Type of Antibiotics	Brand of Products	Type of Use	Group
Amikacin	Amikacin, Amiosin	Injection	Aminoglycosides
Amoxicillin	Amoxicillin, Amoxsan	Syrup, pill	Beta-lactam
Amoxicillin, Clavulanat	Co Amoxiclav, Capsinat	Syrup, pill	Beta-lactam
Azitromycin	Azomax, Infimycin, Zibramax, Zistic	Syrup, Pill, Injection	Macrolide
Ampicillin, Sulbactam	Bactesyn	Injection dan Tablet	Beta-lactam
Cefadroxy	Cefradoxyl, Cefat, Renasistin	Syrup, Pill	Cephalosporins
Cefixime	Cefixime, Fixacep, Nucef, Starcef	Syrup, Pill, drop	Cephalosporins
Cefuroxime	Anbacim	Injection	Cephalosporins
Cefotaxime	Cefotaxime, Biocef, Kalfoxim	Injection	Cephalosporins
Cefoperazone	Cefoperazone, Cefoject	Injection	Cephalosporins
Ceftriaxone	Broadced	Injection	Cephalosporins
Ceftazidime	Ceftazidime, Ceftum, Zibac	Injection	Cephalosporins
Ceftizoxime	Ceftizoxime	Injection	Cephalosporins
Ceftriaxone	Ceftriaxone, Elpicef	Injection	Cephalosporins
Clindamycin	Clindamycin	Pill	Clindamycin
Cotrimoxazol	Cotrimoxazol	Syrup, Tablet	Sulfonamides
Ciprofloxacin	Ciprofloxacin, Baquinor, Renator	Pill	Quinolones
Doxicyclin	Doxicyclin	Kapsul	Tetracycline
Erytromycin	Erytromycin, Erysanbe	Pill, Syrup	Macrolide
Kanamycin	Kanamycin	Injection	Aminoglycosides
Levofloxacin	Cravit, Volequin	Tablet, Infus	Quinolones
Lincomycin	Lincomycin	Pill, Syrup	Lincosamide
Metronidazole	Farnat	Infus	Nitroimidazole
Meropenem	Merem, Merofen, Meropenem	Injection	Beta-lactam
Netilmicin	Netromycin	Injection	Aminoglycosides
Ofloxacin	Ofloxacin, Pharflox	Pill	Quinolones
Primadex	Primadex	Pill, Syrup	Sulfonamides
Tetracycline	Tetracycline, Tetrasanbe	Pill	Tetracycline
Thiamphenicol	Thiamphenicol, Biothicol	Pill	Thiamphenicol

**Fig. 2.** Chromatogram of Ciprofloxacin

antibiotic Ciprofloxacin was still detected. Hamjinda *et al.* (2015) who conducted a research in Bangkok, Thailand found Kuinolon, Ofloxacin, Levofloxacin, Norfloksasin, Sulfametoksazol, Norfloksasin dan Ciprofloxacin. Mater *et al.* (2014) found Ciprofloxacin, Tamoxifen and Cyclo-phosphamide

in France. Furthermore, Varela *et al.* (2014) who investigated the sample from hospital in Portugal, detected Amoxicillin, Ciprofloxacin, Fluoroquinolones, Arsenic, Mercury, Metracyclines, Sulphonamides, and Penicillin G. Santos *et al.*, (2013) detected antibiotics such as Ciprofloxacin,

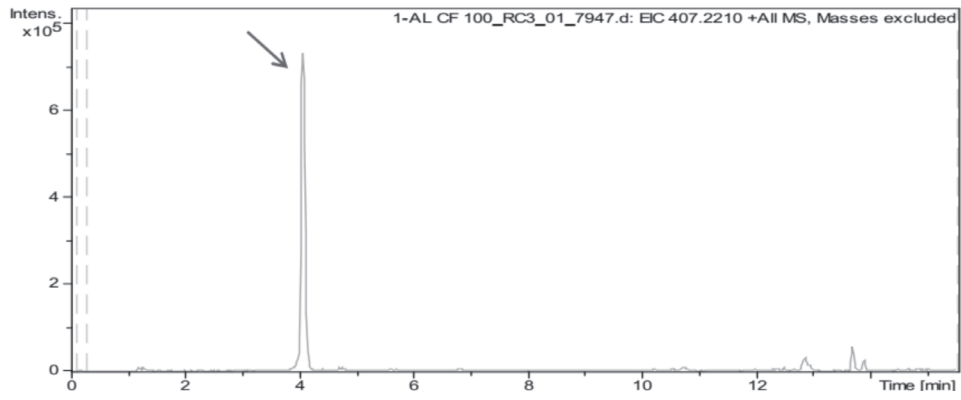


Fig. 3. Chromatogram of Lincomycin

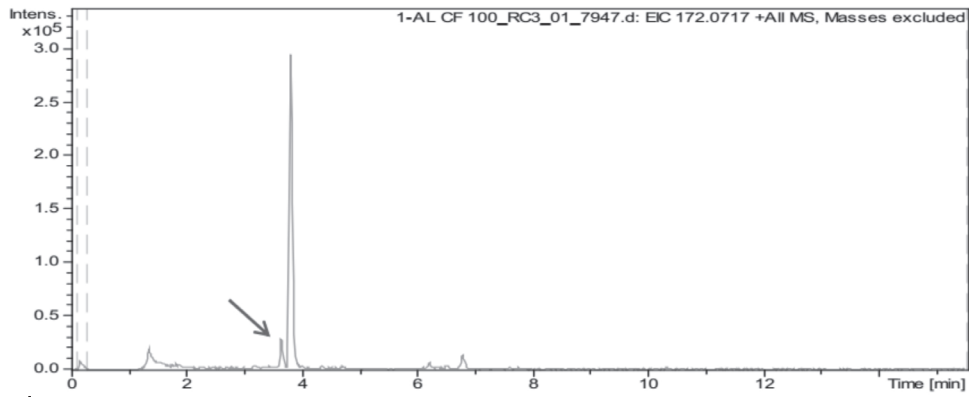


Fig. 4. Chromatogram of Metronidazole

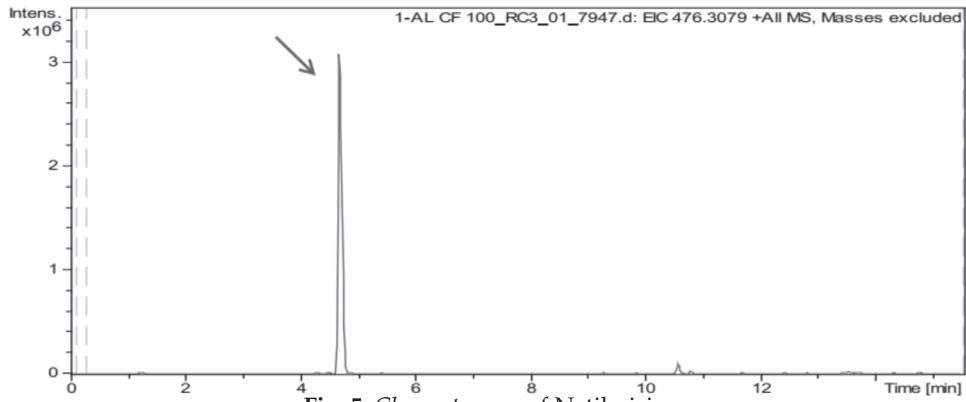


Fig. 5. Chromatogram of Netilmicin

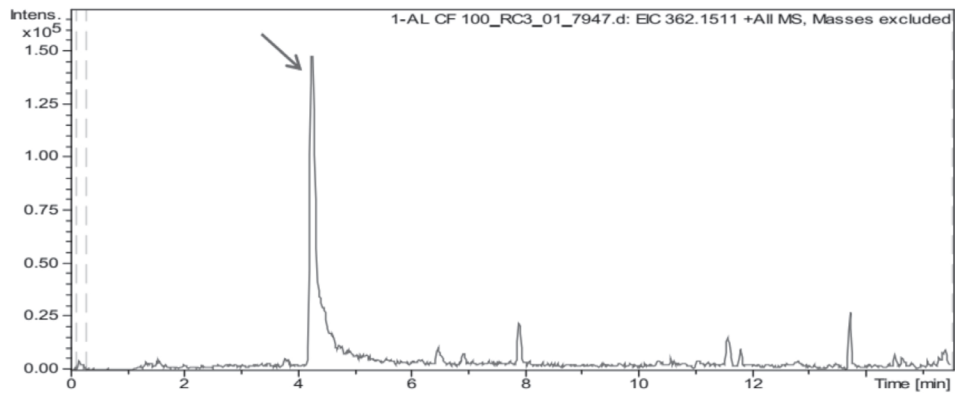


Fig. 6. Chromatogram of Ofloxacin/Levofloxacin

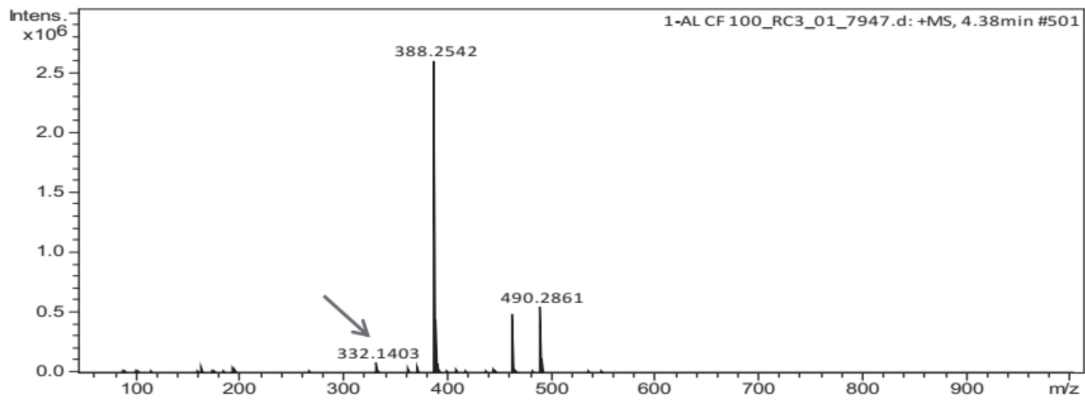


Fig. 7. Spectrum of Ciprofloxacin

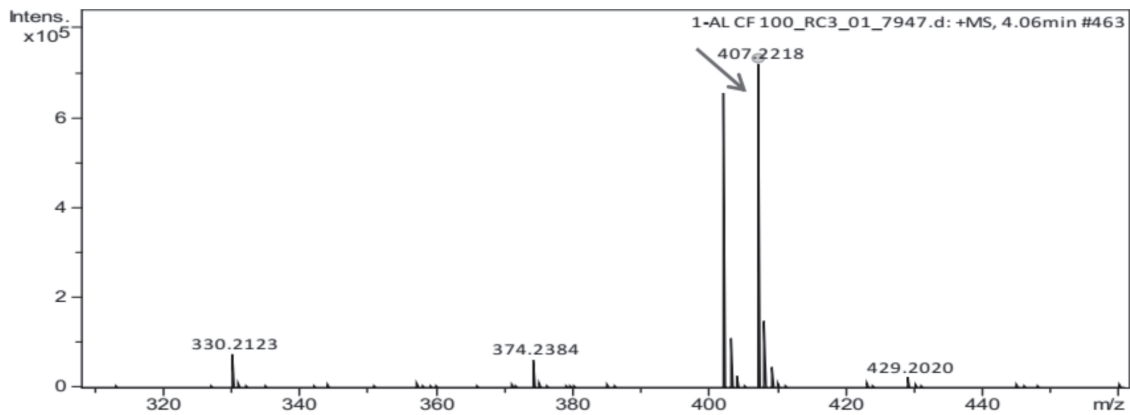


Fig. 8. Spectrum of Lincomycin

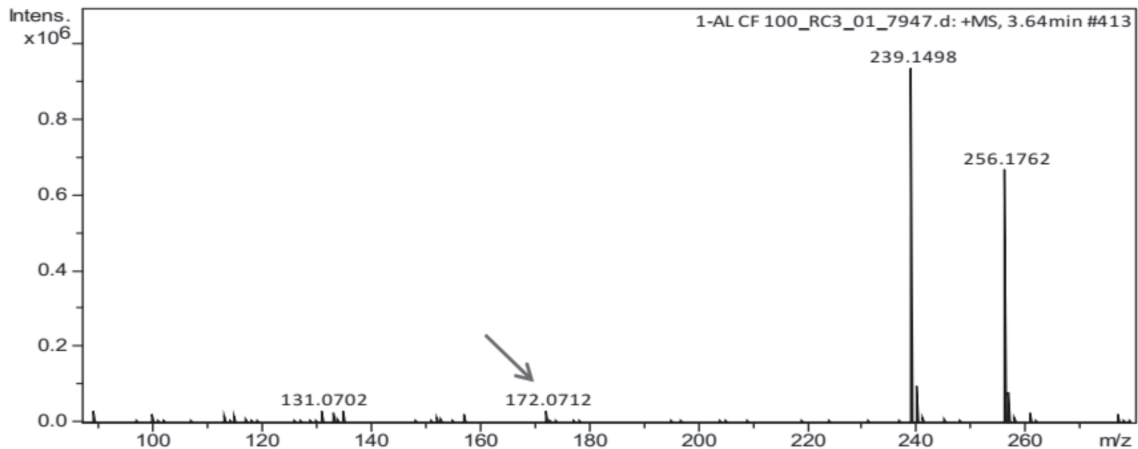


Fig. 9. Spectrum of Metronidazole

Table 2. The Results of Antibiotics Detection in the Wastewater Sample of Hospital X

Antibiotics	RT	Mass (m/z)	MW	Err/mDA	mSigma	Intenstiy	CF
Ciprofloxacin	4,38	$C_{17}H_{19}FN_3O_3^{1+}$	332,140496	0,2	9,4	9,00E+04	100
Lincomycin	4,06	$C_{18}H_{35}N_2O_6S^{1+}$	407,221034	-0,7	10,9	8,00E+05	100
Metronidazole	3,64	$C_6H_{10}N_3O_3^{1+}$	172,071668	-0,2	12,1	3,00E+04	100
Netilmicin	4,74	$C_{21}H_{42}N_5O_7^{1+}$	476,307875	1,4	14,5	3,00E+06	100
Ofloxacin/Levofloxacin	4,25	$C_{18}H_{21}FN_3O_4^{1+}$	362,151061	-0,4	16,8	1,50E+05	100

Ofloxacin, Sulfamethoxazole, Azithromycin, Clarithro-mycin, Acetaminophen and Ibuprofen in Spain.

Identification of Ciprofloxacin detected in the hospital wastewater was due to the level of Ciprofloxacin use rate is very high. Ciprofloxacin has a broad spectrum, active against both negative and positive gram-bacteria. Ciprofloxacin is used for the treatment of respiratory tract infections, urinary tract, gastrointestinal and abdominal infections, including infections by negative gram-bacteria (*Escherichia coli*, *Haemophilus influenza*, *Klebsiella pneumoniae*, *Legionella pneumophila*, *Moraxella catarrhalis*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*) and gram-positive (*Staphylococcus aureus*, *Streptococcus pneumonia*, *Staphylococcus epidermis*, *Enterococcus faecalis*, and *Streptococcus pyogenes*).

Results of laboratory examination of the content of some antibiotics in hospital wastewater showed that Ciprofloxacin has the highest intensity 9×10^4 . Intensity displays the estimation of the amount of Ciprofloxacin concentration as the highest antibiotic compounds in hospital wastewater. Considering the err-value (MDA) of the next data and Sigma, Ciprofloxacin has the smallest value that can be inferred that level of sensitivity and selectivity of detection of these compounds are better.

CONCLUSION

Hospital activities have the potential to generate waste water containing antibiotics. The rest of antibiotics use in health services of the hospital will be mixed with other remaining waste products which are then included into wastewater treatment plants and caused a negative impact on the environment. Early detection of identifying antibiotics contained in the hospital wastewater is required to provide input and suggestion to the regulations applicable in Indonesia. Agilent 6540 Q-TOF/LC /MS is an instrument that has a selectivity and excellent sensitivity to identify qualitatively untargeted antibiotics. There are 5 types of antibiotics detected in hospital wastewater where Ciprofloxacin has the highest concentration estimation due to the intensity value of these antibiotics.

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