Antimicrobial Resistance in Animal as One Health Challenge (Case Study: Drug Dossier Preparation Made from Ciprofloxcacin, Enrofloxcacin, and Flumequine)

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Antimicrobial Resistance (AMR) is critical health issues today. One of factors triggering the increasing incidence of AMR is irrational used of antibiotics in humans and animals. AMR in humans also related to the incidence of AMR in animals, especially to livestock due to the use of Antibiotic Growth Promotors with a sub-therapeutic level. This study aimed to describe the result of drug content assays; product A, B, and C on drug registration dossier from Company X and to relate the impact on public health of AMR incidence. This study used descriptive analysis and laboratory assays of two drug samples using HPLC and one drug sample using spectrofotometer at Pharmacy Laboratory on August - October 2015. Results found two samples (A and B) did not meet the standards; Product A (ciprofloxcacin) and Product B (enrofloxcacin) each obtained at 33.76 g/kg and 41.88 g/kg; while Product C (flumequine) met standard at 219.45 mg/mL (90-110%). The distribution of drugs which was not in accordance with standard was an important factor to the occurrence of antibiotic resistance.

Keywords*: antibiotic growth promotor; anti-microbial resistance; ciprofloxcacin; enrofloxcacin flumequine; one health*

INTRODUCTION

One of the goals to be achieved in Sustainable Development Goals (SDGs) is to ensure a healthy life and encourage prosperity for all people in all ages. The approach in addressing of public health problem at this time is with the One Health concept. It emphasizes that human health associated with the environment and animal health (CDC 2016). The critical health issue which needs one health concept is antimicrobial resistance (AMR).

The use of antimicrobials for the treatment of infectious diseases is growing rapidly since the 19^{th} century until today, and it is followed by the increasing incidence of resistance to antimicrobials. It has been known as many as two million people in the United States each year get a serious infection of bacteria which are resistant and at least 23,000 people dead as a direct result of the infection (CDC 2013). In Indonesia (2008), 23% of bacteria resistant to antibiotics with sample of patients in Soetomo Hospital. Data in 2002 showed that all isolates from blood has a high level of multi resistance to antibiotics and 45-56% of the irrational use of antibiotics (Akalin 2002).

One of factors triggering the increasing incidence of AMR is unwise use of antibiotics in humans and animals. AMR in humans cannot be separated from the incidence of AMR in animals, especially to livestock as a result of the use of feed additives livestock as antibiotic growth promoters (AGP) to the level of sub-therapeutic to be one of the reasons for the burgeoning of bacteria population which is resistant to an-antimicrobials (Barton 2000).

Antimicrobial is widely used as AGP in animal feed around the world to spur growth of livestock in order to grow bigger and faster to prevent infection (Mitchell et al. 1998; Van den Bogaard et al. 2000). Some antimicrobials widely used as AGP are from the class of tetracycline, penicillin, macrolides, linkomisisn, and virginiamycin (Angulo et al. 2000).

In Indonesia, regulation of drug registration of animals for biological and pharmaceutical preparations is in the Act Number 9 of 2009. The regulation concerning the Registration of Veterinary Drugs Standard Operating Procedures, the Director General of Livestock Production Number 55/TN.260/KPTS/DEPTAN/2001 on Animal Drug Registration Application Form which consists of attachment A to L.

Several types of antibiotics commonly used in humans and animals are ampicillin, chloramphenicol, and ciprofloxcacin. Ciprofloxacin is antibiotics of flouroquinolon class where the main working mechanisms is the sub units of *DNA gyrase*; enzyme which plays the important role in the replication of DNA and leads to functional impairment of bacteria. Ciprofloxacin actively eradicates *enterobacteriaceae* and gram-negative rods, such as *Pseudomonas aeruginosa*, *Mycoplasma* is also very good to eradicate *E. coli*. Ciprofloxacin has a wide spectrum compared to the previous quinolones generation like flumequine. Antibacterial ciprofloxacin has the same spectrum as other quinolones generation like enrofloxacin. While flumequine is the first generation fluoroquinolone group of antimicrobials which are used primarily for the treatment of the gastrointestinal tract of livestock (FAO 2016).

The problems associated with the use of antimicrobials in animals have the potential biological hazard; transfer resistance genes from animals to humans (antimicrobial resistance) and chemical hazard (antimicrobial residues) in animals. Residue can be formed due to excessive use and not pay attention the downtime. All of the above was also associated with the production of antimicrobial drugs from pharmaceutical manufacturers.

The objective of this paper was to describe and compare the results of laboratory tests of drugs A, B, and C of the Pharmaceutical Company X to the regulatory standards of government and its relation with risk factors for Antimicrobial Resistance, described the incidence of resistance to antimicrobials with drugs that are not standardized, and describes the importance of the concept of One Health approach to face the challenges of Antimicrobial Resistance.

METHOD

This study used descriptive method and experimental design with laboratory testing by using *High Performance Liquid Chromatography* (HPLC) to determine levels of active ingredients of ciproflocacin, enrofloxcacin, and flumequine using spectrophotometry in the manufacture of drugs A, B, and C in Pharmaceutical Company X.

The principle of the use of HPLC tool is separation of analytics based on polarity. When a sample to be tested is injected into the column, the sample will then be broken down and separated into chemical compounds (analytics) in accordance with different affinities. The separation results will then be detected by the detector (UV spectrophotometer, fluorometer or refractive index) at a particular wavelength, result from the detector subsequently is recorded by a recorder that normally can be displayed using the integrator.

While the spektrofotometer is a tool that consists of spectrometers and photometer. Spectrophotometer produce beams of a specific wavelength spectrum and the photometer is a device to measure light intensity which is transmitted or absorbed, so spectrophotometer was used to measure the relative energy if the energy is transmitted, reflected, or emitted as a function of wavelength.

2.1 Testing time

Period of testing was conducted on August - September 2015 at the Laboratory of Pharmacy Faculty of Pharmacy, University of Pancasila. Some steps were performed as the following:

2.2 Sample preparation

Preparation of drug samples A, B, and C of about 500 grams to ciprofloxcacin, enrofloxcacin, and flumequin of approximately 100 ml, accompanied CoA (Certificate of Analysis) of each the active ingredient. Tests in the laboratory were by using HPLC. The steps of tests performed referring to the Indonesian Pharmacopoeia Veterinary Drugs (FOHI) 2009 as follows:

a. Liquid Chromatography Method:

Test solution: Weigh enrofloxcacin equivalent to 5 mg, add 100 ml of acetonitrile. Strain and if necessary dilute. Standard solution: At 5 mg enrofloxcacin standard, add 100 ml of acetonitrile. Strain and dilute if necessary. Column: C-18 5 μm, size 25 cm x 4.6 mm or appropriate. Kinetic phase: Mix 1 volume of acetonitrile, 10 volumes of acetic acid 5% v/v and 6 volumes of methanol. Detector: Spectrophotometer at a wavelength of 254 nm and 330 nm. Injection: 20 µl of each solution. Identification: (Reference: FOHI vol.2). In the assay, retention time peaks in the chromatogram obtained with solution (1) and solution (2) are same. Assays: (Reference FOHI vol.2) performed by liquid chromatography. Solution (1): Dissolve and dilute the dosage; equivalent to 2 grams of ciprofloxacin to the concentrations of 0.05% w/v. Then Filter it by using paper Whatman GF/C. Solution (2): Standard ciprofloxacin hydrochloride, 0.058% w/v in the kinetic phase. Injection: 5 µL of each solution. Column: Nucleosil 120-C18 40° C. Flow rate: 1.5 ml/min. Kinetic phase: Mix 13 volumes of acetonitrile and 87 volumes of orthophosphoric acid 0.245% w/v, adjust pH 3 with triethylamine. Detector: Spectrophotometer at a wavelength of 278 nm.

RESULTS

Table 1. Result Assays of Ciprofloxacin (Drug A)

Test Parameter	Result	Requirements
Description	White powder	White powder
Ciprofloxacin Content	33,76 g/kg	90-110%

Sample Acceptance Date : August 5^{th}, 2015

Sample Code : K-339/Qlab/VIII/2015

Testing method : High Performance Liquid Chromatography

Table 2. Result Assays of Enrofloxcacin (Drug B)

Test Parameter	Result	Requirements
Description	White powder	White powder
Enrofloxcacin Content	41,88 g/kg	90-110%

Sample Acceptance Date : August 5^{th}, 2015

Sample Code : K-338/Qlab/VIII/2015

Testing method : High Performance Liquid Chromatography

Table 3. Result Assays of Flumequine (Drug C)

Test Parameter	Result	Requirements
Description	Yellow powder	Yellow powder
Flumequine Content	219,45 mg/mL	90-110%

Sample Acceptance Date : August 5th, 2015

Sample Code : K-330/Qlab/VIII/2015

Testing method : spectrophotometry

Based on the test results using HPLC, Table 1 showed that drug A (ciprofloxacin) has a content of 33.76 g/kg, Table 2 for drug B (enrofloxcacin) had a content of 41.88 g/kg, while Drug C (flumequine) in Table 3 had a content of 219.45 mg/mL.

DISCUSSION

Based on the results above, two types of drug with the active ingredient ciprofloxacin and enrofloxcacin did not meet the standard set by the government, namely the minimum levels that must be met is 90% and the maximum acceptable levels is 110%.

Both drugs A and B are class of drugs with sub therapeutic dose levels because it contains the composition of substandard materials. One of factors that causes the test results are not as expected is level of raw materials from the beginning that is already not appropriate.

Data in 2010 showed that 79% of *E. coli* strains were resistant to ampicillin, in which 30% of strains were resistant to ciprofloxacin. In the United States (1999-2000) occurred in 43% of cases *Sthapylococcus aureus* infections resistant to methicillin. Some harmful bacteria are also resistant to giving antimicrobial (*Mycobacterium tuberculosis* and *Pseudmonas aureginosa* (Kuswandi 2012). If an animal drug that is not standardized (example: drug A and B) was massively produced and successfully marketed to consumers (farmers) illegally then it could be an opportunity to be risk factor for resistance antimicrobial and one example of how antimicrobial resistance can spread to humans is through consumption of food of animal origin. If it is not handled properly, it can spread to humans.

Non-compliance of a pharmaceutical company in applying good and true principles of making drug (CPOHB) is also a risk factor that could improve the circulation of veterinary medicines which are not standardized to the consumers. This is influenced by supervision factor, monitoring, surveillance and sanctions. A holistic approach is needed to fight the antimicrobial resistance. One health approach is defined as collaborative effort of various multi disciplines, working locally, nationally, and globally to achieve optimal health for people, animals and the environment (Avma 2008). It is recognized that human health associated with animal health and the environment. One health concept on antimicrobial resistance could be one right choice. By the fact that the antimicrobial agent is essential for healing infections in humans and animals. Antimicrobial resistance does not recognize geographic boundaries of humans and animals. increase antimicrobial resistance incidence in humans relate to incident in animals (FAO, WHO, OIE Factsheet). There are three essentials in health competencies, namely leadership, governance and partnership. One health workers need to have both a strong disciplinary background as well as training to work in multi-disciplinary settings. There may be people formally tasked with one health duties. Often, one health duties may be fulfilled by someone working without explicit recognition of this role in their job description. Human resource development should support, encourage and sustain assignment that build one health perspective and attributes; assist personnel to develop and apply one health perspective; and recognize and reward individuals and teams that perform well in such roles.

A key role for One Health governance is to sustain relationship over the longer term. Productive, trusting, and mutually rewarding partnership takes time to build and require support. Legal frameworks, physical, virtual infrastructure, and the system of budgeting should support collaborative and coordinated policy, planning research, and action. Ease of transferring funds between agencies or organizations to realize One Health goals is an important mechanism for success. Institutional cultures should foster transparent information sharing, surge capacity for issues needing collaboration, and effective communication for coordinated messaging. Sharing workload and facilities (for example one rather than two central laboratories to meet the public health needs of the veterinary services and the Ministry of Health may be a means to not only increase the efficiency of delivery of One Health programs but also to build understanding and trust across programs.

Antimicrobial assistance is a good example in which partnerships amongst academia, animal health, public health, and environment agencies are as vitally important collaborations with citizens and private sector players who are often in the best position to know is required in terms of allocation of resources, benefits and the management of risks and their impacts (Stephen 2016).

CONCLUSION

Distribution and use of veterinary medicines which are not in accordance with the standards is risk factor of antimicrobial resistance. The wise use is necessary to reduce as much as possible the use of antimicrobial selection pressure on humans, animals and plants. The approach in addressing of this problem is with the One Health concept. It emphasizes that human health associated with the environment and animal health.

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REFERENCES

Akalin, E. 2002. The evolution of guidelines in an era of cost containment. Surgical prophylaxis. *J Hosp infect*.

Angulo, F.J., J.A. Nunnery and H.D. Blair. 2004. “Antimicrobial resistance in zoonotic enteric pathogens.” *Rev. Sci. Tech. Off. Int. Epiz.* 23(2): 485-496.

AVMA. 2008. “One Health: A New Professional Imperative.” *One Health Initiative Task Force Final Report*. Schaumburg, IL: American Veterinary Medical Association.

Barton, M.D. 2000. “Antibiotic use in animal feed and its impact on human health.” *Nutrition Research Reviews*. 13 (2): 1-19.

Direktorat Jenderal Peternakan dan Kesehatan Hewan. 2013. Farmakope Obat Hewan Jilid II Indonesia. Jakarta.

Mitchell, J, MW Griffiths, SA Mcewen, WB Mcnab, and AJ Yee.1988. “Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance.” *Journal of Food Protection*. 61(6):742-56.

Nisi, R, Brink, N, Diaz, F, and Moulin, G. 2013. “Antimicrobial use in animals: Analysis of the OIE Survey on Monitoring of the Quantities of Antimicrobial Agents Used in Animals.” *World Organisation for Animal Health*. Paris

Timothy F. Landers, RN, *et al*. 2012. “A Review of Antibiotic Use in Food Animals : Perspective, Policy, and Potensial.” *Public Health Report.*

https://www.cdc.gov/onehealth/about.html [Accessed 6 September 2016]

https://www.cdc.gov/onehealth/about.html [Accessed 6 September 2016]

http://www.oie.int/eng/A_AMR2013/Presentations/S2_4_Fran%C3%A7oisDiaz.pdf. [Accessed Sept 6, 2016].

http://www.oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/FAO_OIE_WHO_AMRfactsheet.pdf [Accessed October 28, 2016].

Stephen, C., & Stemshorn, B. (2016). "Leadership, governance and partnerships are essential One Health competencies." *One Health*, *2*, 161-163.