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Antibiotics resistant *Escherichia coli* isolated from aquatic ecosystems in Palembang, South Sumatra, Indonesia

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Abstract. *Verawaty M, Apriyani N, Tarigan LR, Apriyan ET, Laurenta WC, Muharani. 2020. Antibiotics resistant* Escherichia coli *isolated from aquatic ecosystems in Palembang, South Sumatra, Indonesia. Biodiversitas 21: 86-97.* Antibiotics-resistant bacteria (ARB) are one of the emerging water contaminants currently gaining serious global concern due to their adverse risk to ecosystems, wildlife, and public health. This study determined antibiotics resistant *Escherichia coli* isolated from aquatic ecosystems in Palembang. Most probable number (MPN) was used for bacterial estimation and the Kirby-Bauer method was used for susceptibility test against antimicrobial agents. The results indicated that 82% of *E. coli* isolates from 28 sampling sites were resistant to ampicillin, 57% to tobramycin, and 71% to tetracycline. The isolates showed intermediate profile to kanamycin (50%), 57% to cotrimoxazole, 50% to cefixime, and 54% to gentamycin. These isolates still showed sensitivity towards ciprofloxacin (86%) and chloramphenicol (61%). Total coliform (TC) numbers ranged from 0 to >1600 MPN/100 mL. Sampling sites with high MPN values of ≥1600 MPN/100 mL were Sekanak watersheds (SW1, SW8, SW11, SW12, SW13, and SW14) followed by SW2 and SW9 with a value of 1600 MPN/100 mL, while samples from cattle and fish farms (CW) varied from 0 to 170.000 MPN/100 mL. TC of samples collected from retention ponds (RP) ranged from 0 (RPJSC) to 1.600.000 MPN/100 mL (RPSH3). The *Escherichia coli* (EC) counts varied from 1.7 x 10^3 (RPSH2) to ≥1.6 × 10^4 MPN/100 mL (SW11, SW12, SW13, and SW14). Several samples (SW3, RPPI, RPTS, RPSB, RPIBA, and RPOPI) have no *E. coli*. The results indicated some of the sampling locations that exceeds the quality standard of water have been regulated by the Governor of South Sumatra and the Indonesian Government.

Keywords: *Escherichia coli*, antibiotics resistant bacteria, aquatic ecosystems

INTRODUCTION

Fecal coliform is an indicator of contamination of human or animal feces in water bodies. If fecal coliform is in high concentration in a site, it is very likely that pathogenic organisms are also present (Mishra et al. 2018). Understanding emerging contaminants in the water are important because water is the link among the four major ecosystems-human, animal, soil, and aquatic-involved in the circulation of antibiotic resistance (Nwosu, 2001; Baquero et al. 2008). Currently, antibiotic-resistant bacteria (ARB) are becoming one of the emerging water hazards (Pruden et al. 2006; Taylor et al. 2011). Aquatic ecosystems play an ecological and evolutionary role in driving the persistence emergence and spread of antimicrobial-resistant (AMR) microorganisms (Taylor et al. 2011). A study was done by Ribeiro et al. (2012) showed *Escherichia coli* antibiotic resistance patterns and its groundwater contamination origin in France, this study contributed to the understanding of the emerging issue. Wang et al. (2019) recently suggested that horizontal gene transfer (HGT) is one of *E. coli* capabilities potentially harm our ecosystem, wildlife, and public health; especially its prevalent in biofilms and sediment, this is due to its ability on genetic exchange with potential to increase the spread of environmental pathogens. They also suggested it has been more ARB and antibiotics resistant genes (ARGs) were detected in residential than in hospital wastewater (Li et al. 2015), nanomaterials (Qiu et al. 2012), disinfectants (Guo et al. 2015), disinfection by-products (Li et al. 2016) and ionic liquids (Luo et al. 2014); the ionic liquid is suggested taking roles in the spread of ARGs by promoting HGT. The genetic material in resistant microorganisms can be passed from one to another and create unavoidable spreads of contamination. Jain (2019) reported that the Ganga river had been polluted by a large number of resistant antibiotics bacteria which was suspected from some waste discharged from households, drug manufacturing units, hospitals, and poultry industries. Another study was done by Cho et al. (2018) suggested that the other possible human's coliform sources include sewage leaks, failing septic tanks, municipal, residential, medical, and industrial waste facilities and also animal sources include runoffs from animal farms, land application of animal manure, pet wastes from parks, and wildlife; all of them are potentially increase the spreads of the hazardous contaminants.

The increased spread of bacteria that are resistant to several types of antibiotics or "superbugs" can be due to various human activities such as excessive used of antibiotics in human, livestock and fish farming, poor infection control in health care systems, poor hygiene and sanitation in developed and developing countries which resulted in approximately 700.000 deaths each year

(Guyomard-Rabenirina et al. 2017). Antibiotic resistance causes hundreds of thousands of deaths annually (Review on Antimicrobial Resistance 2014), so this is a major global health threat with an increasing trend (WHO 2014). Ballantyne (2007) suggested that some of today's human activities potentially trigger the AMR such as some households' antibacterial contained products, and antibacterial agent such as triclosan is reported as one of the stressors that potentially develop bacterial defense and tolerance for their survival and antibiotics resistant community reproducibility through cross-resistance and genetic mutations.

Coliform is known as one of the largest groups of bacteria in bacterial polluted water (Mishra et al. 2018). In some countries, where the incidence of antibiotic resistance among both coliform and fecal coliform bacteria from seawater and shellfish samples is high, the coliform bacteria are regarded as dangerous pollution indicators (Grabow et al. 1974). Regular monitoring of coliform levels in the environment provides the status of water potability, warns prior incidence of various public health concerns, and paves the way for designing remedial measures (Mishra et al. 2018). Currently, prevalence of multi-drug resistance in the coliforms triggered by the increasing of antimicrobials used in household, clinical, veterinary, animal husbandry and agricultural settings including from fertilized fields that lead to more severe and complex antibiotic contamination is current global concern; therefore, the potential antibiotic contamination sites require additional attention (Zhang et al. 2014). High incidence of antibiotic resistance among gram-negative bacteria due to the widespread and uncontrolled used of antibiotics in agriculture and medicine has been documented (Anderson 1968; Richmond 1975). The evidence of antibiotic-resistant coliform from effluents and land runoff enter marine receiving waters has also been reported (Smith 1970, 1971; Grabow et al. 1974). Antimicrobial agents are currently widely used to promote growth and disease control in animals. The number of veterinary antibiotics (VAs) reaching 105-106 tones annually (Levy 1998; Sarmah et al. 2006; Li et al. 2013), and antibiotics that poorly absorbed in the animal gut can cause around 40-90% of the antibiotics excreted via urine or feces in the form of origin compounds or metabolites (Kumar et al. 2005; Gutiérrez et al. 2010). The residue of VAs in animal wastes has been widely reported (Martinez-Carballo et al. 2007; Zhao et al. 2010; Motoyama et al. 2011; Pan et al. 2011; Li et al. 2013). Evidence of hospital settings and the retail food supply as the sources of the spread of antibiotic resistance enteric bacteria (Gorbach 2001; Karlowsky et al. 2003; Edge and Hill 2005) and those emerging contaminants enter and spread in the rivers and lakes which are served as sources of drinking water, recreation, or irrigation.

According to Mishra (2018), antibiotics entered and dispersed into our environment through seepages, sewages, clinical or agricultural settings runoffs and it can create antibiotic resistance genes pools. Their presences in below minimum inhibitory concentration (MIC) affect their transfer, assimilation, and propagation caused multiple antibiotic resistances (MAR) index of bacterial isolates from environment. Some studies describe the possible origin of the antibiotics and antibiotics resistant genes (ARGs) in the environment; and suggested there is a relationship between anthropogenic contamination with residual antibiotics (RAbs) that spread, accumulate and disseminate in the aquatic environments (Baquero et al. 2008; Gillings 2013). ARGs in the aquatic environments correlate with human activities and antibiotic usage (Pei et al. 2006; Pruden et al. 2012; Khan et al. 2013). Furthermore, antibiotic usage in humans, veterinary medicine, and the pharmaceutical industrial sites, especially their wastes, are responsible for the increasing prevalence of antimicrobial-resistant bacteria (AMRB) (Holmberg et al. 1987; Cheng et al. 2012; Adegoke et al. 2017).

Awareness of the potential contaminants in the aquatic environment in Palembang that can threaten the ecology of the city, public health, and wildlife is urgently needed, and the river as one of the water resources for the city water supply has to be maintained for its sustainability. Therefore, this study aimed to investigate the potency of antibiotic resistance in *E. coli* isolates from aquatic ecosystems in Palembang. This information is expected to contribute to the understanding of antibiotic resistance in the aquatic environment and the potential environmental and public health risks associated with them. The current study aims to highlight the alarming issue of the prevalence of multiple antibiotic-resistant *E. coli* in the aquatic system of Palembang, which is a potent environmental and public health concern.

MATERIALS AND METHODS

Sampling locations

Surfaces water samples for microbiological assays were collected from 41 sampling sites, i.e., cattle and fish farms, retention ponds, and non-point sources of domestics disposal along Sekanak River watersheds and its tributaries in Palembang City (Figure 1). Samples were collected by a purposive random sampling method from March to May 2018. All samples were collected in sterile containers and immediately placed in iceboxes and maintained at 4°C before reaching the laboratory and analyzed directly for measuring biological parameters, while physical and chemical parameters were directly measured on-site as per standard methods for the examination of water and wastewater (APHA 1995). Water quality parameters were measured in situ using a Lutron YK-2005WA instrument; they were water temperature (°C), pH, total dissolved solids (TDS in mg/L), and dissolved oxygen (% saturation and mg/L). Preserved water samples were analyzed for total Nitrogen (N tot in mg/L) (TOC-N-Analyzer-Teledyne Tekmar), total Phosphorus (P tot in mg/L) (SNI 06-2483-1991), chemical oxygen demand (COD in mg/L) (SNI 6989.2:2009) and total suspended solids (TSS in mg/L) (SNI 06-6989.3-2004). The characteristics of locations of each of the sampling site were presented in Table 1.

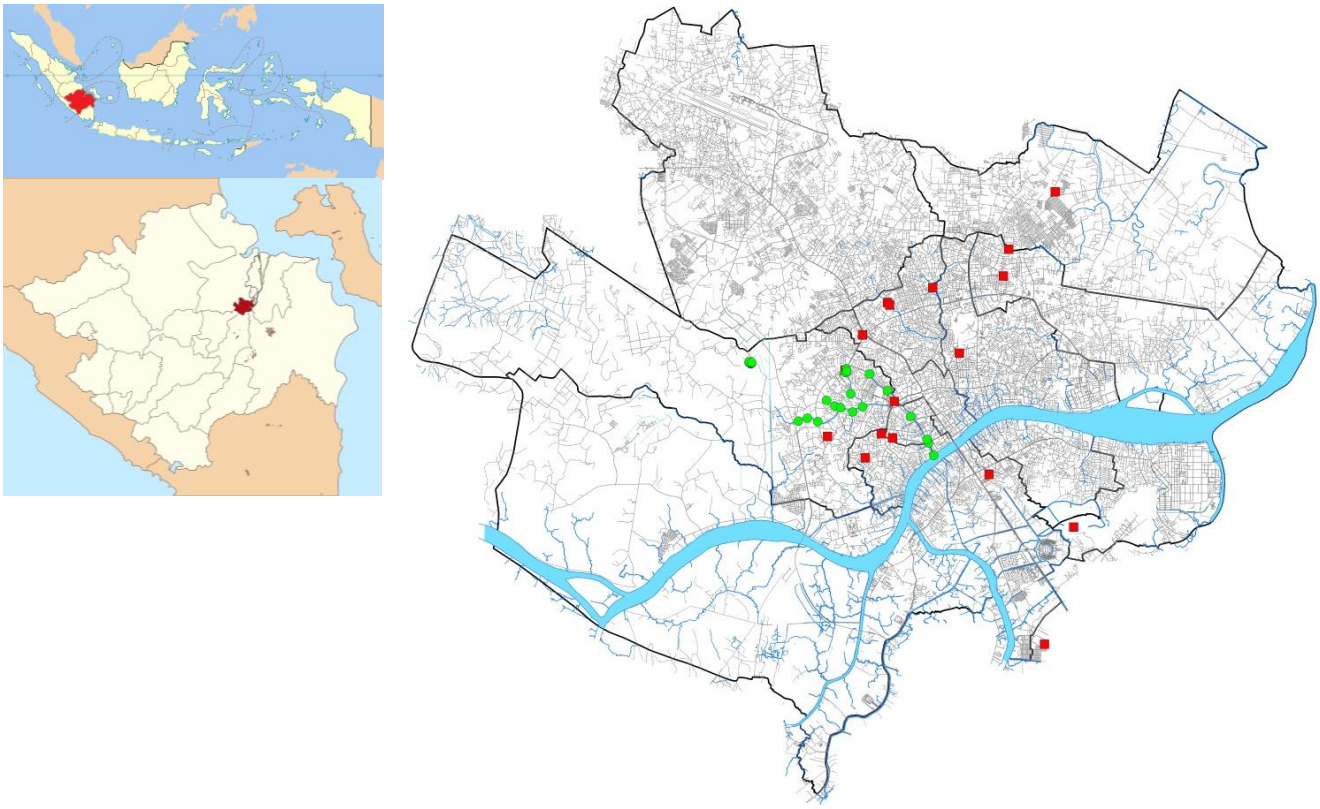

Figure 1. Research locations of water sampling sites in Palembang City, South Sumatra, Indonesia

Total coliform enumeration and *Escherichia coli* identification

Most Probable Number (MPN) method was used to estimate the total coliform in water samples according to SNI Method (SNI 2897-2008 and SNI 01- 2897-1992): Presumptive Test was conducted by pipetting 10 mL water samples from each sampling points to a 30 mL screw-cap tube (5 tubes for each sample) containing 10 mL of Double Strength Lactose Broth (DSLB). Similarly, add 1 mL of the same samples to 5 tubes of 17 mL screw-cap tube containing 7 mL of Single Strength Lactose Broth (SSLB) and 0.1 mL of each sample to 5 screw-cap tubes with 7 mL Lactose Broth Single Strength. All the tubes were incubated at 36 ± 1ºC for 48 hours. The number of tubes with the presence of gas formation is the positive presumptive test. Confirmation Test was carried out on tubes that give positive results on Lactose Broth in the presumptive test. The sample showed a positive result was added to a tube containing 10 mL of Brilliant Green Lactose Broth (BGLB), and then incubated at 36 ± 1ºC for 48 hours. The presence of gas in the tube containing BGLB confirms the presence of coliform bacteria in the sample. The number of positive tubes forming gas was recorded. Then the results were compared to MPN tables. Eijkman Test: The Eijkman test is a specific test for identifying fecal coliform at 44-46ºC. It was performed by inoculating the positive culture on the Lactose Broth media into a tube containing EC Broth media in Durham tubes. The tubes were incubated in a water bath at 44-45ºC for 48 hours. Positive results were indicated by gas formation in the Durham tube, then the samples were determined for the density of thermotolerant fecal coliform. The completed test was performed on positive cultures to determine the presence of *E. coli* by streaking on EMBA plates and then incubated at 36ºC for 48 hours. Colonies with green metallic sheen were inoculated on the oblique NA medium and incubated at 35ºC for 24 hours. After the colony grew on the NA media, then IMViC test was done to confirm the presence of *E. coli*. The indole test was done by inoculating the colony in Tryptone Broth media and incubated at 35ºC for 24 hours. After 24 hours incubation, the indole reagent was added to each tube and shaken for 10 minutes. The crimson ring on the surface showed a positive indole reaction. Methyl Red Test was done by inoculating the colony in NA into the MR-VP Broth and incubated at 35ºC for 48 hours. After 48 hours, 4 to 5 drops of methyl red were added. The presence of red color showed a positive reaction. Voges Proskauer Test was done by the inoculating colony in NA into the MR-VP. Then they were incubated at 35ºC for 48 hours. After 48 hours, 0.6 mL alpha naphthol solution and 0.2 mL of KOH solution were added and homogenized then left for 2-4 hours. The positive reaction was indicated by pink to dark red result. Citrate Test was done by inoculating colony in NA into Simmons Citrate Broth and then incubated at 35ºC for 48 hours. The positive reaction was shown by blue color, and the negative reaction was green color.

Antibiotics susceptibility test

Susceptibility tests for antimicrobial agents were conducted using the Kirby-Bauer method according to Clinical and Laboratory Standards Institute (CLSI 2010) and Urase and Sato (2016). All of *E. coli* strains were pre-screened for their ability to form colonies on Mueller-Hinton agar supplemented with selected antimicrobial agents at the minimum inhibitory concentrations (MICs) listed in the Clinical Laboratory Standards Institute (CLSI, 2010) for Enterobacteriaceae of resistant strains, i.e. tetracycline 10 µg/mL, ciprofloxacin 5 µg/mL, kanamycin 30 µg/mL, cotrimoxazole 30 µg/mL, tobramycin 10 µg/mL, chloramphenicol 30 µg/mL, cefixime 5 µg/mL, gentamycin 10 µg/mL, and ampicillin 10 µg/mL. The strains growing on one or more plates containing antimicrobial agents were examined for their susceptibility to nine antimicrobial agents by the Kirby-Bauer method.

Escherichia coli strains were inoculated in nutrient broth and incubated at 35+2°C for five hours. The inoculum was used within 30 minutes of preparation (Andrews (2001). The colonies were touched with a loop, and then the growing colonies were transferred into a sterile broth. The turbidity of suspension was adjusted to a 0.5 McFarland standard (EUCAST 2003). The McFarland standard was prepared by adding 0.5 mL of 0.048 M $BaCl_2$ (1.17 % w/v $BaCl_2.2H_2O$) to 99.5 mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring. Cotton swabs were used for streaking the diluted broth onto Mueller-Hinton agar plates. After air drying, antibiotic discs were placed on agar plates. The plates were inverted and incubated aerobically at 35+2 °C for 16 to 18 hours. The zone of inhibition was measured and interpreted according to the CLSI (CLSI, 2010). The ATCC strain of *E. coli* was used as a control strain. Preparations of antibacterial agents were conducted according to EUCAST (2003); the stock solutions were made by using Formula F.1A and F.1B as follows:

$$\text{(F.1A)}\quad \text{weight of powder (mg)} = \frac{\text{volume of solution (mL) x Concentration } (\frac{\text{mg}}{\text{L}})}{\text{Potency of powder } (\frac{\text{mg}}{\text{g}})}$$

$$\text{(F.1B)}\quad \text{volume of diluent (mL)} = \frac{\text{Weight (mg) x Potency } (\frac{\text{mg}}{\text{g}})}{\text{Concentration } (\frac{\text{mg}}{\text{L}})}$$

$$\text{(F2.)}\quad D = \frac{(DV - DC) + (DH - DC)}{2}$$

Where:
DV: Vertical diameter
DC: Disk diameter
DH: Horizontal diameter

The diameters (in millimeters) of the clear zones as growth inhibition around the antimicrobial agent diskswere measured following the method described by Sayah (2005). Diameter of the inhibitory zone (two quadrants) were measured according to Formula 2 (F2). Diameter of inhibitory zone was interpreted according to Clinical and Laboratory Standard Institute (CLSI 2012) as follows: ampicillin (sensitive > 17 mm; intermediate: 14-16 mm; resistant < 13 mm); tetracycline (sensitive ≥ 19 mm; intermediate: 15-18 mm; resistant ≤ 14 mm); cefixime (sensitive > 19 mm; intermediate: 16-18 mm; resistant < 15 mm); chloramphenicol (sensitive ≥ 18 mm; intermediate: 13-17 mm; resistant ≤ 12 mm); ciprofloxacin (sensitive ≥ 21 mm; intermediate: 16-20 mm; resistant ≤ 15 mm); cotrimoxazole (sensitive ≥ 16 mm; intermediate: 11-15 mm; resistant ≤ 10 mm); canamycin (sensitive ≥ 18 mm; intermediate: 14-17 mm; resistant ≤ 13 mm); gentamycin (sensitive ≥ 15 mm; intermediate: 13-14 mm; resistant ≤ 12 mm); tobramicin (sensitive ≥ 18 mm; intermediate: 13-17 mm; resistant ≤ 12 mm).

RESULTS AND DISCUSSION

The sampling sites in this study are presented in Figure 1 and Table 1; namely 18 retention ponds (RP), lake (1 site), seven sites of cattle ranches and fish ponds (CW1-CW7) and 15 sampling sites located along the Sekanak River and its tributaries namely Muhajirin River and Baung River (SW1-SW15). Sekanak River is one of the Musi River tributaries, and a tourist center in Palembang, South Sumatra. Some of Palembang residents live surrounds the Sekanak River. The characteristics of the sampling sites are described in Table 1 and presented in Figure 2.

The quality standard of river water quality in South Sumatra has been regulated through South Sumatra Governor Regulation No.16 of 2005, which classified river water into 3 classes based on water quality for its provisions. Also, there was Government Regulation No. 8 of 2012 concerning the quality standards of liquid waste from industrial activities, hotels, hospitals, domestic and coal mining. In this regard, effluent standards for domestic wastewater are further regulated through two products of law at the ministry level; Ministerial Regulation No. 5 of 2014 (Permen No. 5) regulates general quality standards for liquid waste disposals and Ministerial Decree No. 112 of 2013 (Kepmen No. 112) which specifically regulates effluent standards for domestic wastewater. The effluent parameters of domestic wastewater based on the two regulations as follows: pH-6-9, BOD 100 mg/L, TSS 100 mg/L, and oil and Fat 10 mg/L. Characteristics of water samples (physical and chemical) taken at each sampling site were presented in Table 2; which includes 8 parameters (pH, temperature, total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), dissolved oxygen (DO), N total, and Ptotal).

The collected water samples showed that pH values at almost all samples still met the quality standards (pH 6-9), but only the CW6 sample had a low pH (3,2). Sampling sate of CW6 was a new fish pond opened in swampy-land with acidic water conditions. The total suspended solids (TSS) in several locations exceeded the quality standard of 50 mg/L (CW1, SW11, SW 14, RPKIB, RPKIK, RPPI, RPBKN, and RPBrimob), with the highest TSS value was sample of SW11 (178 mg/L), while the TSS value of other locations were below the standard, ranged from 6 to 48 mg/L). The concentration of total dissolved solids (TDS) ranged from 39 to 545 mg/L. High TDS values were observed at CW locations (280 to 545 mg/L), and the

lowest was measured in SW15 sample (39 mg/L), followed by OPI Lake (47 mg/L) and RP IBA (59 mg/L). The other locations showed TDS values ranged from 89 to 223 mg/L. All of these values are still below the specified quality standard (1,000 mg/L). Sample collected from OPI Lake had high DO value (8.78 mg/L) while the other locations were below the quality standard value that has to meets a minimum of 6 mg/L, ranged from 0.49 to 5.84 mg/L. For surface water, the maximum limit (10 mg/L). The results showed that COD values of all samples exceeded the maximum limit of the quality standard value, with the highest COD values was the sample collected from cattle farms, ranged from 198 to 222 mg/L, while the COD value of other locations ranged from 13 to 91 mg/L.

Figure 2. Some of the sampling sites in this study, in Palembang City, South Sumatra, Indonesia. Locations of SW11 and SW12 sampling sites (A and B); SW6, SW9 and SW5 sampling sites (C, D, and E); SW13, SW3 and SW8 sampling sites (F, G, and H); RPKM (I), RPSH (J) and RPJSC (K).

Table 1. Characteristics of water sampling sites in Palembang City, Indonesia

Site ID	Sampling sites characteristics
CW1-CW3	Cattle and fish farms, closed pond
CW4	Small-scale poultry farm closed pond
CW5	Cattle farms, fish farms, closed pond.
CW6	Originally swamp area, new open pond, low pH
CW7	Cattle farms, fish farms, closed pond
SW1	The residential settlement, sewage overflowed.
SW2	The residential settlement, small-scale poultry farm, sewage overflowed
SW3	Densely population settlement, domestic solid and liquid wastes disposal, affected by the daily tidal stream
SW 4	Domestic solid and liquid wastes disposal, affected by the daily tidal stream
SW5	Poor sanitation, domestic wastes disposal, sewage overflowed, FOG, presence of animal carcasses and animal feces, affected by the daily tidal stream
RPSH1-2	Human-made closed pond, moderately algal growth, sewage overflowed, smelly water
RPSH3	Human-made closed pond, relatively transparent water, domestic wastes disposal, sewage overflow, residential and road runoff
SW6	Moderately algae bloom, domestic wastes disposal, sewage overflow and affected by daily tidal
SW7	Home scale cattle farm, faulty septic tank, sewage overflow, moderately algae bloom, domestic wastes disposal and affected by daily tidal
SW8-9	Moderately algae bloom, domestic wastes disposal, sewage overflow and affected by daily tidal
SW10	No solid and liquid domestic waste disposal, flow and shallow water stream
SW11-12	Sekanak traditional market, dense population, domestic wastes disposal, sewage overflow, data were collected before the renovation works were started
SW13-14	City business area, dense population, near hotels, food street, and shopping malls, sewage overflow
RPKM	Natural pond connected to marsh ecosystem, poor sanitation area, domestic waste disposal, many trees (open area), residential area
RPKIB	Human-made, sport and recreational spots, city garden, residential area, flow water, surrounded by trees
RPKIK	Human-made, recreational spot, city garden, residential area, closed pond, surrounded by trees
SW15	Sekanak traditional market, dense population, domestic wastes, sewage overflow
RPPI	Human-made, less dense population, road runoff, shopping mall, ponds with continuous aeration, stagnant throughout the year, less domestic waste disposal, fewer trees (open area), shallow water pond, data were collected before the renovation works were started
RP Polda	Human-made, less dense population, road runoff, shopping mall, stagnant throughout the year, sewage overflow, fewer trees (open area)
RPAst1-2	Human-made, Poor sanitation, dense population, closed pond
RPSP	Human-made connected to marsh ecosystem, poor sanitation, dense population, stagnant throughout the year, less domestic waste disposal, fewer trees (open area), shallow water pond
RPSM	Human-made connected to marsh ecosystem, less dense population, stagnant throughout the year, less domestic waste disposal, fewer trees (open area), shallow water pond
RPTS	Natural pond connected to marsh ecosystem, less dense population, stagnant throughout the year, domestic waste disposal, fewer trees (open area), shallow water pond, wastes of tempeh industry
RPSB	Human-made, dense population, stagnant throughout the year, close to the highway, domestic waste disposal, many trees (open area), deep water pond
RPIBA	Human-made, less dense population, stagnant throughout the year, no domestic waste disposal, very few trees (open area), shallow water pond, a newly re-excavated dam
RPBKN	Human-made, poor sanitation area, far from the highway, stagnant throughout the year, solid domestic waste disposal, almost no trees, traditional market, deepwater pond
RPJSC	Natural swampy pond, part of the sports area, no domestic wastes disposal, stagnant throughout the year.
OPI Lake	Human-made, closed pond, sedimentation swamp pond, affected by traditional market runoff and domestic wastes disposal
RP Brimob	Natural pond connected to marsh ecosystem, recently renovated, data were collected before the renovation works were started

Note: *) NT: Not tested; CW: Cattle Farm ; SW: Sekanak watershed; RP: Retention pond; RPSH: RP_near St Khodijah Hospital; RPKM: Kemang Manis. RPKIB: Kambang Iwak Besak; RPKIK: Kambang Iwak Kecik; RPPI: Palembang Icon; RPAst: RP Kemuning; RPSP: Seduduk Putih; RPSM: Sapta Marga; RPTS; Tanjung Sari; RPSM: Sematang borang; RPIBA/Bangau; RPBKN; RPJSC: Jaka Baring Sport Center; OPI Lake: Ogan Permata Indah; RPBrimob.

Nitrogen total of the samples ranged from <0.10 to 4.86 mg/L. All samples from cattle farms (CW) showed high Ntot values (2.75-4.84 mg/L), while the lowest was the RPTS sample. Ntot values from other samples ranged from 1.32 to 4.23 mg/L. The Phosphate total (Ptot) of the samples ranged from 0.72 mg/L (the highest was CW1) to < 0.01 mg/L (the lowest was OPI Lake). The maximum allowable level of phosphate content based on the quality standard is set at 0.2 mg/L. The concentrations of Ptot from CW samples were mostly high (0.33-0.72 mg/L). The other locations have the Ptot values below the standards limit are RPKIB and RPKIK (0.09-0.1 mg/L), followed by RPPI and

RP Polda (both were 0.01 mg/L). Results of the total coliform (TC) of samples collected from several aquatic environments showed TC ranged from 0 to >1600 MPN/100 mL. Sampling sites with the ≥1.600.000 MPN/100 mL were TC of SW1, SW8, SW11, SW12, SW13, and SW14 were ≥ 1.600.000 MPN/100 mL (Table 3.) The South Sumatra Governor Regulation No 16 of 2005 for river water quality standard limited Coliform total at 10.000 MPN/100 mL.

The validation results indicated the presence of *E. coli* was negative in the samples of SW3, RPPI, RPTS, RPSB, RPIBA, and RPOPI. Colonies that showed positive blue-green metallic in EMBA media (*E. coli*) were subcultured several times to obtain pure isolates of *E. coli and* to be used will be further tested for antibiotics susceptibility test. To confirm the species of bacteria so that the IMViC test was carried out include indole test, methyl red test, Voges-Proskauer test, and citrate test. *E. coli* bacteria will produce indol and positive of methyl red positive as well as Voges-Proskauer and negative of citrate tests in the IMViC test. In general, fecal coliform consists of three bacterial genera, including *Klebsiella pneumonia*, *E. coli*, and *Enterobacter sp*. All of these three bacteria ferment lactose and produce acids and gases at 44ºC; however, only the *E. coli* colonies show a green metallic color on EMB agar so that *E. coli* can be differentiated from other genera. This, study only focuses on exploring the multiresistant antibiotics *E. coli*. A list of samples containing *E. coli* presented in Table 3.

Table 2. Physical and chemical characteristics of water samples collected from 41 sampling sites in Palembang City, Indonesia

Samples codes	X_COR	Y_COR	PH	Temp (ºC)	DO (ppm)	TDS (mg/L)	COD	Ntot	Ptot	TSS
CW 1	467261	9671863	7.6	29.2	0.65	545	222	4.86	0.72	68
CW 2	467261	9671863	7.2	30.2	0.70	458	201	4.52	0.64	22
CW 3	467261	9671863	7.1	30.3	0.75	414	201	4.32	0.58	35
CW 4	467261	9671863	7.4	30.4	0.86	280	110	2.72	0.41	25
CW 5	467261	9671863	7.2	30.5	0.77	335	142	3.12	0.33	32
CW 6	467261	9671863	3.2	30.6	0.76	333	133	3.56	0.44	7
CW 7	467261	9671863	6.4	30.7	0.49	440	198	4.25	0.51	21
SW 1	469686	9670699	6.7	30.8	0.93	152	55	3.12	0.22	19
SW 2	469947	9670519	7	30.9	4.17	168	60	3.24	0.29	23
SW 3	470122	9670472	7.3	30.10	4.95	161	37	3.88	0.56	51
SW 4	470811	9670498	7.4	30.11	4.24	186	83	3.57	0.37	13
SW 5	470502	9670338	7.5	30.12	2.64	203	60	4.23	0.41	25
RPSH 1	470299	9671690	7.4	30.13	5.69	196	61	3.77	0.29	48
RPSH 2	470271	9671570	7.2	30.14	4.61	223	91	2.97	0.34	8
RPSH 3	470318	9671612	7.1	30.15	4.65	113	83	4.46	0.49	19
SW 6	469088	9670140	7.4	30.16	5.84	180	67	4.38	0.34	18
SW 7	468802	9670053	7.3	30.17	2.98	171	35	3.11	0.73	17
SW 8	470439	9670907	7.4	30.18	4.13	192	41	3.42	0.61	16
SW 9	469413	9670034	7.4	30.19	5.84	181	37	3.18	0.48	18
SW 10	471027	9671525	7.5	30.20	5.84	195	44	3.26	0.72	10
SW 11	473035	9668979	7.3	30.21	5.02	154	30	3.71	0.75	178
SW 12	472870	9669367	7.4	30.22	5.28	176	33	3.89	0.84	10
SW 13	472320	9670197	7.3	30.23	4.84	201	52	2.28	0.73	10
SW 14	471587	9671001	7.4	30.24	4.84	189	48	3.16	0.48	51
RPKM	470899	9668904	7.6	27.5	3.46	166	45	3.12	0.22	33
RPKIB	471752	9669524	7.3	27.7	3.31	89	47	4.82	0.09	62
RPKIK	471402	9669654	7.3	27.4	3.31	98	40	4.55	0.1	54
SW 15	472825	9669481	7.3	28.8	3.05	39	37	4.22	0.89	48
RPPI	471810	9670665	7.4	27.5	4.09	125	41	4.58	0.01	74
RPPo	470813	9672747	7.4	27.2	1.6	145	31	3.36	0.12	39
RPAst 1	471592	9673759	7.5	28	2.23	147	30	4.15	0.18	43
RPAst 2	471659	9673682	7.4	27.4	1.49	181	30	3.39	0.08	39
RPSP	473007	9674227	7.9	28.5	1.26	113	80	2.52	0.02	32
RPSM	475217	9674588	7.7	28.8	5.13	91	48	1.12	0.09	11
RPTS	475381	9675425	7.5	31.7	1.12	128	13	<0.1	0.12	47
RPSB	476833	9677224	7.7	30.7	4.76	121	31	1.32	0.11	22
RPIBA	473842	9672173	7.9	30.2	2.05	59	15	1.32	0.08	45
RPBKN	474765	9668380	7.7	30	3.98	113	41	4.22	0.09	60
RPJSC	477411	9666737	5.1	29	5.91	140	13	2.16	0.06	6
OPI Lake	476498	9663078	7.3	29.9	8.78	47	25	3.13	<0.1	19
RPBRM	469717	9669569	7.4	31.8	3.41	112	36	3.12	0.29	51

Note: *) NT: Not tested; CW: Cattle Farm ; SW: Sekanak watershed; RP: Retention pond; RPSH: RP_near St Khodijah Hospital; RPKM: Kemang Manis. RPKIB: Kambang Iwak Besak; RPKIK: Kambang Iwak Kecik; RPPI: Palembang Icon; RPAst: RP Kemuning; RPSP: Seduduk Putih; RPSM: Sapta Marga; RPTS; Tanjung Sari; RPSM: Sematang borang; RPIBA/Bangau; RPBKN; RPJSC: Jaka Baring Sport Center; OPI Lake: Ogan Permata Indah; RPBrimob.

Table 3. Most Probable Number (MPN) and IMViC of water samples

Codes	Total coliform (MPN/100 mL)	*E. coli* (MPN/100 mL)	IMViC Result
CW 1	4.9×10^4	2.0×10^3	(+) *Escherichia coli*
CW 2	1.4×10^4	-ve	-ve
CW 3	3×10^5	4.2×10^4	(+) *Escherichia coli*
CW 4	1.7×10^5	1.6×10^4	(+) *Escherichia coli*
CW 5	3.5×10^5	2.0×10^3	(+) *Escherichia coli*
CW 6	-ve	-ve	-ve
CW 7	9.2×10^5	1.7×10^4	(+) *Escherichia coli*
SW 1	$\geq 1.6 \times 10^5$	$\geq 1.6 \times 10^4$	(+) *Escherichia coli*
SW 2	1.6×10^5	7.9×10^4	(+) *Escherichia coli*
SW 3	9.2×10^5	2.0×10^3	-ve
SW 4	3×10^5	3.4×10^4	(+) *Escherichia coli*
SW 5	3×10^5	1.7×10^4	(+) *Escherichia coli*
RPSH 1	7.9×10^4	-ve	-ve
RPSH 2	5.4×10^5	1.7×10^3	(+) *Escherichia coli*
RPSH 3	1.6×10^5	9.2×10^3	(+) *Escherichia coli*
SW 6	7.9×10^5	7.0×10^3	(+) *Escherichia coli*
SW 7	2.4×10^5	1.7×10^4	(+) *Escherichia coli*
SW 8	$\geq 1.6 \times 10^5$	1.6×10^4	(+) *Escherichia coli*
SW 9	1.6×10^5	1.6×10^4	(+) *Escherichia coli*
SW 10	9.2×10^4	1.6×10^3	(+) *Escherichia coli*
SW 11	$\geq 1.6 \times 10^5$	$\geq 1.6 \times 10^4$	(+) *Escherichia coli*
SW 12	$\geq 1.6 \times 10^5$	$\geq 1.6 \times 10^4$	(+) *Escherichia coli*
SW 13	$\geq 1.6 \times 10^5$	$\geq 1.6 \times 10^4$	(+) *Escherichia coli*
SW 14	$\geq 1.6 \times 10^5$	$\geq 1.6 \times 10^4$	(+) *Escherichia coli*
RPKM	1.7×10^5	4.0×10^3	(+) *Escherichia coli*
RPKIB	2.0×10^3	2.0×10^2	(+) *Escherichia coli*
RPKIK	9.2×10^5	2.0×10^3	(+) *Escherichia coli*
SW 15	2.2×10^4	7.0×10^3	(+) *Escherichia coli*
RPPI	1.7×10^5	3.4×10^4	-ve
RP Polda	3×10^5	1.7×10^4	(+) *Escherichia coli*
RPAst 1	1.7×10^4	2.0×10^3	(+) *Escherichia coli*
RPAst 2	5.4×10^5	1.3×10^4	(+) *Escherichia coli*
RPSP	5.4×10^5	7.0×10^4	(+) *Escherichia coli*
RPSM	9.2×10^3	-ve	-ve
RPTS	9.2×10^5	1.6×10^4	-ve
RPSB	1.7×10^5	3.4×10^4	-ve
RPIBA	3.3×10^3	-ve	-ve
RPBKN	2.7×10^4	4.0×10^3	(+) *Escherichia coli*
RPJSC	-ve	-ve	-ve
OPI Lake	2.3×10^4	8.0×10^3	-ve
RP Brimob	NT*	NT*	-ve

Note: *) NT: Not tested; CW: Cattle Farm ; SW: Sekanak watershed; RP: Retention pond; RPSH: RP_near St Khodijah Hospital; RPKM: Kemang Manis. RPKIB: Kambang Iwak Besak; RPKIK: Kambang Iwak Kecik; RPPI: Palembang Icon; RPAst: RP Kemuning; RPSP: Seduduk Putih; RPSM: Sapta Marga; RPTS; Tanjung Sari; RPSM: Sematang borang; RPIBA/Bangau; RPBKN; RPJSC: Jaka Baring Sport Center; OPI Lake: Ogan Permata Indah; RP Brimob.

Table 4. Susceptibility of *Escherichia coli* strains collected from sampling sites to several antibiotics

Sample codes	Antibiotics susceptibility*)								
	Tbr	Amp	Tet	Can	Cyp	Cot	Cef	Gen	Chm
Ec_CW 1	S	S	R	I	S	S	S	S	S
Ec_CW 3	S	S	R	I	S	S	S	S	S
Ec_CW 4	S	S	R	S	S	S	S	S	S
Ec_CW 5	S	S	R	S	S	I	I	S	S
Ec_CW 7	R	S	R	I	S	I	S	S	S
Ec_SW1	S	R	R	S	S	S	S	S	S
Ec_SW2	S	R	R	I	S	S	S	S	S
Ec_SW4	R	R	R	R	S	I	S	S	S
Ec_SW5	R	R	R	S	S	R	R	S	R
Ec_SW6	R	R	R	S	S	I	R	S	S
Ec_SW7	R	R	R	I	S	S	S	S	S
Ec_SW8	R	R	I	S	S	S	S	I	S
Ec_SW9	R	R	R	I	S	I	I	I	S
Ec_SW10	S	R	I	S	S	I	I	S	S
Ec_SW11	R	R	R	I	S	S	I	I	S
Ec_SW12	R	R	I	R	R	R	R	I	I
Ec_SW13	R	R	S	R	R	R	I	I	I
Ec_SW 15	R	R	I	I	S	R	R	I	S
Ec_SW14	R	R	R	I	I	I	S	I	I
Ec_RPSH2	R	R	R	S	S	I	I	I	S
Ec_RPSH3	R	R	R	R	S	I	I	S	I
Ec_RPKM	R	R	I	I	S	I	I	I	I
Ec_RPKIB	R	R	I	I	S	I	S	S	S
Ec_RPKIK	R	R	I	S	S	I	I	I	I
Ec_RP Po	R	R	R	I	S	I	I	I	S
Ec_RP_Ast 2	R	R	R	S	S	I	I	I	I
Ec_RPTS	R	R	R	I	S	I	I	I	I
Ec_RPBKN	R	R	R	I	I	I	I	I	I

Note:*) R: Resistant, I: Intermediete, S: Sensitive, Tbr: Tobramicin, Amp: Ampicillin, Tey: Tetracyclin, Can: Kanamycin, Cyp: Ciprofloxacin, Cot: Cotrimoxazole, Cfx: Cefixime, Gen: Gentamycin, Chm: Chloramphenicol

There were 1422 colonies from 41 sampling sites in this study. Based on the results of the verification test, it showed 22% of those colonies were positively *E. coli* (n: 307); 15% of *E. coli* strains were susceptible to antibiotics. Amongst those of 22% isolates, 8% were collected from cattle farms, 18% were isolated from retention ponds, and 74 % were isolated from Sekanak River watersheds and its tributaries, canals, creeks, and channels. Antibiotic susceptibility test was carried out using the disk diffusion method against *E. coli* strains that had been grown on the Mueller-Hinton Agar. The antibiotic inhibitory zone of *E. coli* strains from 41 locations in this study showed varying degrees of sensitivity to the various types of antibiotics used in the test. Bacteria tested were categorized as sensitive, intermediate and resistant based on their inhibitory scores according to the standard interpretation of inhibition zone diameters, which were determined by the Clinical Laboratory Standards Institute (CLSI, 2012). Isolates that showed *E. coli* (+) were tested for antibiotics susceptibility to 9 antibiotics (tobramycin, ampicillin, tetracycline, kanamycin, ciprofloxacin, cotrimoxazole, cefixime, gentamycin, and chloramphenicol) (Table 4).

The results of antibiotics susceptibility test of the *E. coli* strains indicated that 75% of the *E. coli* strains were resistant to tobramycin, 82% to ampicillin, 71% to tetracycline, 14% to kanamycin, 7% to ciprofloxacin, 14% to cotrimoxazole, 14% to cefixime, and both 4% to gentamycin and chloramphenicol. The results indicated 25% of *E. coli* strains showed intermediate result to tetracycline, 50% to kanamycin, 7% to ciprofloxacin, 57%

to cotrimoxazole, 46% to cefixime, 50% to gentamycin and 32% chloramphenicol, while sensitive results showed that 25% of *E. coli* sensitive to tobramycin, 18% to ampicillin, 4% to tetracycline, 36% to kanamycin, 86% to ciprofloxacin, 29% to cotrimoxazole, 39% to cefixime, 46% to gentamycin and 64% to chloramphenicol. Based on the percentage of resistant and intermediate sensitivity to multiple antibiotics, it was concluded that *E. coli* strains collected from RPSH3 were high resistance *E.* to multiple antibiotics followed by the other five high resistance strains, which were Ec_SW5, Ec_SW6, Ec_SW8, Ec_SW12, Ec_SW13, and Ec_RPSH3. The *E. coli* isolates with a high percentage for resistant to antibiotics and also showed intermediate response to multiple antibiotics were Ec_SW9, Ec_SW14, Ec_RPSH3, Ec_RPKM, Ec_RPBKN, and Ec_RPTS. Lastly, the sensitive *E. coli* strains to antibiotics ranged from 11% (the lowest which was the EC isolate from RPBKN that was coded as Ec_RPBKN) to 89% (the highest was isolate Ec_CW4).

The *E. coli* strains from RPs and SWs showed lower sensitivity compared to those from CWs. *E. coli* strains collected from CWs mostly sensitive to antibiotics. This study showed that there were *E. coli* colonies grow in the clear zone of cefixime paper disk (Figure 3). Cefixime is known to have a bacteriostatic effect with the ability to inhibit bacterial growth and reproduction without killing the bacteria; therefore, there will be a clear zone, but the bacteria still grows in the inhibitory zone.

Discussion

This study showed that *E. coli* collected from several aquatic environments had high resistance to tobramycin, ampicillin, and tetracycline. It is in agreement with the result of Kozak et al. (2009) reported that 83% of *E. coli* isolated from swine were resistance to tetracycline, and 58% were multi-resistance. Resistance of *E. coli* to at least two classes of antimicrobial agents in *E. coli* has been frequently found in the environment (Von Baum and Marre, 2005; Young, 1993) and it has been estimated that 17.6% of the genes in *E. coli* has been acquired by horizontal transfer at a rate of 16 kb/Myr (Lawrence and Ochman, 1998). Other studies also reported high rates of *E. coli* resistance to tetracycline and kanamycin (81.4%), chloramphenicol (75.7%), gentamycin (74.3%), and ampicillin (72.9%) (Alhaj et al. 2007). High rates of multi-resistance *E. coli* may be caused by long term use of antibiotics, especially overuse of antibiotics to treat caused by *E. coli*. Fernandez et al. (2013) reported a correlation between the inappropriate use of antibiotics with the percentage of resistance to antibiotics in Manggarai and West Manggarai Regencies-East Nusa Tenggara Timur (NTT).

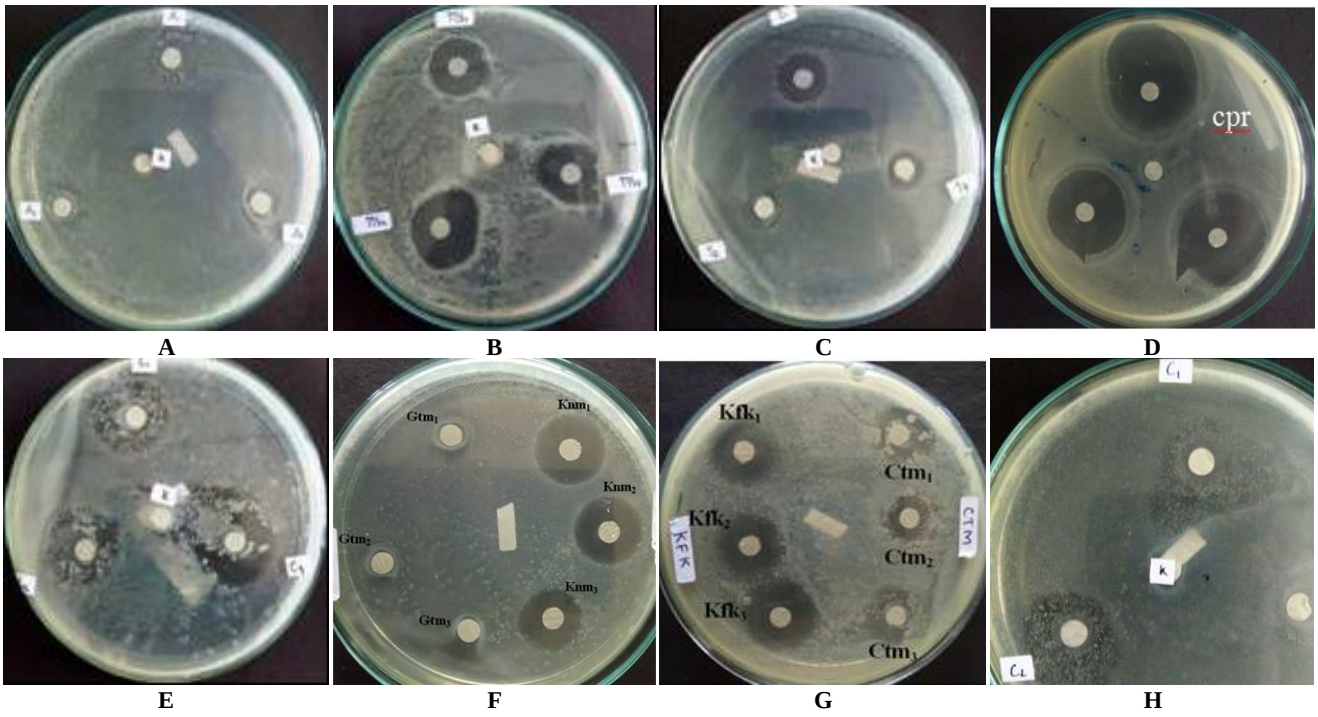

Figure 3. Antibiotics susceptibility tests by Kirby-Bauer method against *E. coli* strains from the aquatic environment in Palembang City, Indonesia. A. Ampicilin, B. Tetracyclin, C. Tobramicyn, D. Ciprofloxacin, E. Cefixime, F. Gentamycin and Kanamycin, G. Chloramphenicol and Cotrimoxazole, H. Cefixime (SW14)

The Indonesian Ministry of Health (2011) states that the widespread use of inappropriate antibiotics raises various problems and is a global threat to health, especially bacterial resistance to antibiotics. It has been suggested as one of the leading causes of *E. coli* resistance to ampicillin. Other studies carried out by Hadi et al. (2013) and Indonesian Ministry of Health (2005) in Indonesia reported that 43 % of 2,494 *E. coli* isolates in Indonesia showed resistant to antibiotics, 34% were resistant to ampicillin and high rates of resistance to tetracycline due to uncontrollably used in medicine and animal husbandry.

The results of this study showed that the isolated *E. coli* was sensitive to ciprofloxacin (86%) according to CLSI interpretation compared to 8 other antibiotics. However, several reports suggest the need for re-evaluation of CLSI breakpoints to prevent further development of fluoroquinolone resistance because several clinical isolates of *E. coli* showed reduced susceptibility to ciprofloxacin (Baudry-Simner et al. 2012) due to its irrational and inappropriate use (Ali et al. 2010). Kibret and Abera (2011) reported that ciprofloxacin was considered suitable for the empirical treatment of *E. coli* in northeast Ethiopia. Ciprofloxacin is one of fluorinated quinolones structurally related to nalidixic acid. It is a broad-spectrum antibiotic, more sensitive to gram-negative bacteria, and less effective against gram-positive bacteria (Campoli-Richards et al. 1988).

The results showed that *E. coli* isolated in this study were sensitive to gentamycin and chloramphenicol. Gentamycin belongs to the aminoglycoside group that has a broad spectrum, especially against gram-negative bacilli infections such as *E. coli*. Gentamycin has the ability to penetrate bacterial walls and bind ribosomes and interfere with the translation process and cause misreading in translating bacterial mRNA (Lintong et al. 2012). This study showed there was a bactericidal effect of cefixime to the isolated *E. coli* which was indicated by bacterial growth in the clear zone of cefixime paper disk. The bactericidal activities of cefixime to *E. coli* and *H. influenza* reported by Somekh et al. (1996). However, Arshad et al. (2012) reported that clinical isolates of *E. coli* and *S. aureus* had developed resistance to cefixime. A study by Ayatollahi et al. (2013) reported that 148 *E. coli* isolates from patients less than 18 years old showed high rates of the bacteria resistance to cefixime (57.9%) and cotrimoxazole. Other study by Sah et al. (2016) in Nepal reported that 72.7% of *E. coli* resistance to ampicillin, followed by cephalexin (59.3%), cotrimoxazole (45.2%), cefixime (40%), ceftriaxone (26.3%), norfloxacin (25.9), ciprofloxacin (25%), ofloxacin (20.7%) nitrofurantoin (9.7%), gentamycin (9.4%) and amikacin (8%). *E. coli* isolated from various sources including human, animals, environments, and other geographic locations showed dynamics and divers susceptibility towards antibiotics. The microbial dynamic changes require constant and regular monitoring for our future health hazards awareness; diseases spread prevention and ecosystem, wildlife and public health protection.

This study showed that sampling sites with low pH of water result in the negative presence of coliform and *E. coli* (CW6, pH 3,2 and RPJSC pH 5,1); however, in this study the correlation of pH and the total of both coliform and *E. coli* were not investigated. Low water pH can affect the respiratory capacity of aquatic organisms including bacteria, and can result in death due to asphyxia (Boyd, C. E. 1992). The highest coliform and *E. coli* numbers were observed in sampling sites with sewage overflows, near traditional markets, with domestic's wastes disposals (SW11, SW12, SW13, and SW14). Most of these sampling sites have high Ptot level. In the sampling sites connected to marsh ecosystem, shallow water streams, surrounded by trees (open green areas) showed high DO compared to other sites with fewer trees. The reduced population of trees and plants along riverbank and streams decreases shading which results in warmer water temperatures, it can lower dissolved oxygen concentrations indirectly because warm water holds less oxygen. Phosphorus is important for organisms, but in excessive concentration, it is harmful to most aquatic organisms such as decreasing DO levels of the water and causing the death of fish and many organisms. Turbidity is an indicator of the number of particles suspended in water that, in high concentrations can damage the habitats of fish and other aquatic organisms (APHA 1995).

In conclusion, this study indicated the evidence of resistant *E. coli* isolates to multiple antibiotics isolated from aquatic ecosystems in Palembang City, the response of the isolates towards the tested antibiotics were varied. In general, the results indicated that 82% of the isolates were resistant to ampicillin, 57% to tobramycin, and 71% to tetracycline. The isolates showed intermediate profile to kanamycin (50%), 57% to cotrimoxazole, 50% to cefixime, and 54% to gentamycin. These isolates still showed sensitivity towards ciprofloxacin (86%) and chloramphenicol (61%). The results also indicated that some of the sampling locations exceed the quality standard of water that has been regulated by the Governor South Sumatra and Indonesian Government. Finally, the *E. coli* isolated from different sources showed dynamics and divers susceptibility towards antibiotics. Therefore constant and regular monitoring is important for ensuring our future health hazards awareness; diseases spread prevention and ecosystem, wildlife and public health protection.

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