Extended spectrum beta-lactamase-producing *Escherichia coli* surveillance in the human, food chain, and environment sectors: Tricycle project (pilot) in Indonesia

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ABSTRACT

The World Health Organization (WHO) has been implementing antimicrobial surveillance with a “One Health” approach, known as the Global Surveillance ESBL *E. coli* Tricycle Project. We describe the implementation of the Tricycle Project (pilot) in Indonesia, focusing on its results, challenges and recommendations. The samples were 116 patients with bloodstream infections caused by ESBL *E. coli*, 100 rectal swabs collected from pregnant women, 240 cecums of broiler, and 119 environmental samples, using the standardized method according to the guidelines. ESBL-producing *E. coli* was found in 40 (40%) of the 100 pregnant women, while the proportion of ESBL-producing *E. coli* was 57.7% among the total *E. coli*-induced bloodstream infections. ESBL-producing *E. coli* was isolated from 161 (67.1%) out of 240 broilers. On the other hand, the average concentration of *E. coli* in the water samples was 2.0 × 10^8 CFU/100 mL, and the ratio of ESBL-producing *E. coli* was 12.8% of total *E. coli*. Unfortunately, 56.7% of questionnaires for patients were incomplete. The Tricycle Project (pilot) identified that the proportion of ESBL-producing *E. coli* was very high in all types of samples, and several challenges and obstacles were encountered during the implementation of the study in Indonesia. The finding of this study have implication to health/the antimicrobial resistance (AMR) surveillance. We recommend continuing this project and extending this study to other provinces to determine the AMR burden as the baseline in planning AMR control strategies in Indonesia. We also recommend improving the protocol of this study to minimize obstacles in the field.

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1. Introduction

Antimicrobial resistance (AMR) has become a global challenge that needs to be addressed. An increase in multidrug resistant microorganisms has been reported in many countries [1–3]. Meanwhile, there have been stagnancies in antimicrobial discovery since 1990. The AMR problem has been affecting economic and public health sectors [4–6]. The World Health Organization (WHO) reported the results of global surveillance of AMR by the country in 2014. In this report, the prevalence of E. coli from bloodstream infections with resistance to cefotaxime and ceftriaxone was 10% and 13.8%, respectively, while Klebsiella pneumoniae resistant to cefotaxime and ceftriaxone was 53.3% and 67.2%, respectively, in Indonesia. However, the prevalence of E. coli that are resistant to 3rd-generation cephalosporins has significantly increased in 5 years. According to Global Antimicrobial Surveillance System (GLASS) data in 2019, more than 50% of E. coli isolates from bloodstream infections are resistant to 3rd generation cephalosporins [4,5]. In Indonesia, nine hospitals participated in GLASS surveillance implementation in 2019, and 16 hospitals participated in 2020.

Multisector activities contribute to the AMR burden; therefore, multisector coordination and cooperation are needed to control antimicrobial resistance. The World Health Organization (WHO) Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR) has been implementing antimicrobial surveillance that uses ESBL-producing E. coli as an indicator. This surveillance uses a “One Health” approach and involves the human, animal (food chain) and environment sectors simultaneously, known as The Global Surveillance ESBL E. coli, Tricycle Project. This pilot project was implemented in some countries such as Ghana, Pakistan, Malaysia, Madagascar, Senegal and Indonesia. As an initial project, all countries that participate should implement at least working packages 1–3 of a total of 7 working packages. The objectives of this surveillance are to establish an integrated surveillance system to monitor ESBL-producing E.coli in three main areas: human, animal (food chain), and the environment across member states; to establish a simple and standardized method to isolate and monitor ESBL-producing E.coli; to compare the prevalence of ESBL-producing E.coli in each of the three sectors among member states; and to have longitudinal system in place to assess the effect of intervention.

In Indonesia, the Tricycle Project (pilot) started at the end of 2018 and consisted of working packages 1, 2, 3, and 5. The implementation of the project in Indonesia was slightly different from that of other countries since the WHO South-East Asia Region (SEARO) had modified this activity by inserting epidemiology data collection and analysis, known as the Epi-X protocol. The combination of the Tricycle-Epi X project was expected to demonstrate the connection between humans, the food chain, and the environment from an epidemiological perspective [7]. This is the first AMR study that involved the Ministry of Health, Ministry of Agriculture and Ministry of Environment and Forestry. This study was a model for the National AMR Surveillance System with a “One Health” approach in Indonesia and expected to be carried out by expanding the coverage area continuously.

Here, we describe the implementation of the Global Surveillance ESBL E. coli Tricycle Project in Indonesia, focused on its results, findings, challenges and recommendations. The recommendations are addressed to the coordinator for the AMR controlling program in three ministries. It is also intended to improve the protocol and the procedure of the implementation of the Tricycle Project to be adapted to manage the problems we encountered in the field. Nationally, the result of the study is of high significance because of its comprehensive nature. The results could be used as baseline data to develop the National Action Plan of AMR across sectors. Globally, the results were comparable to those of other countries that also implemented the Tricycle Project with standardized methods, including species indicator and resistance types. The study implementation could be used as best practice for other countries that have the same AMR problems as Indonesia.

2. Material and methods

This study was conducted in Jakarta, the capital city of Indonesia, from October 2018 to December 2019. The city lies in a lowland area on the northwestern coast of the island of Java. It covers an area of 664.01 km² and is inhabited by approximately 11.1 million people in 2020 [8]. The samples were collected from the human, animal and environment sectors (Fig. 1). Ethical approval extension for this study was obtained from the Ethics Committee of the National Institutes of Health Research and Development, Ministry of Health, Indonesia (No: LB.02.01/2/KE014/2019).

2.1. Samples and sampling sites

Samples for each sector were collected from the same area (Fig. 1) and period. Human samples consisted of blood samples obtained from patients with bloodstream infections in two selected hospitals and rectal swabs obtained from healthy pregnant women who visited a primary health care (PHC) facility for antenatal care (ANC). Samples from animals/food chains were broiler cecums collected from six markets or slaughterhouses. The environment samples were surface water taken from six markets/slaughterhouses, and three up/midstream and three downstream sites. The summary of the sample characteristics is shown in Table 1.

Sampling sites were identified with the assistance of geographical information system (GIS) software (QGIS 3.2 Bonn). Data from patients with bloodstream infections in the previous year were used as baseline data. We then aggregated the number of cases at the sub-district or ‘Kecamatan’ level. The high-risk sub-districts for bloodstream infections were identified. Based on the spatial analysis, most cases lived in East Jakarta. Because of this analysis, this study focused on East Jakarta. Hospital A, Hospital B, and a primary health care (PHC) facility in Jakarta were chosen as sampling sites for human subjects (Fig. 1). According to the Tricycle Guideline, this study required hospitals with a minimum of 5000 with blood cultures per year to have 100 positive E. coli in a year. Hospitals A and B reported >5000 blood cultures each year in total, therefore, these hospitals met the minimum requirement.

In this study, the broiler was chosen as representative of the food chain that does not relate to any religious issues and most sources of ESBL-producing E. coli [9,10] Once the clusters of higher risk in human populations were identified, a spatial analysis was carried out to select the sites within or in the closest proximity to these clusters for sampling in the animals. Based on the spatial analysis, four markets (Pondok Bambu, Sawah Barat, Ciplak, and Deprok) and two slaughterhouses (Pulo Gadung and Rawa Keping) were selected as sampling sites for the animal sector (Fig. 1).

Rivers that cross through or come into close proximity of human cases and identified market sites and slaughterhouses were considered for environmental sampling sites. A total of 12 sites were selected for water sampling (Fig. 1). The sites included rivers, wastewater and canals located upstream (n = 2), midstream (n = 1), downstream (n = 3) of the rivers, market wastewater drains (n = 4), and communal canals near the slaughterhouses (n = 2). The upstream surface water will be considered representative of the pre-urban area and impact in the catchment. Communal waste sites (midstream) will ideally correspond to a treatment plant or a major collecting sewer to represent human fecal material, while waste from the markets/slaughterhouses will represent animal fecal material. Sampling was stratified over the season and collected in the same regions as the human and food chain sampling sites. The study design of the environmental samples was expanded from the Tricycle Guideline and Epi-X protocol. In the Tricycle Guideline, the minimum requirement was eight sites with 6–8 sample collections per site (48–64 samples in total); a total of 119 samples were collected from 12 sites in this study [11].
The sample collection and laboratory examination methods of this study referred to the guidelines of the Tricycle Project. Modifications were made to incorporate the Epi-X method in the epidemiology and to adapt to field conditions. For patients with bloodstream infections, the identification of *E. coli* producing ESBL was carried out in a hospital laboratory according to the procedures established in each hospital (Table 1). A total of 14,682 samples of blood cultures from two hospitals (A and B) were examined from October 2018 to December 2019. The identification of ESBL-producing *E. coli* in Hospital A was carried out using Vitek-2 (bioMérieux) in the hospital laboratory, while in Hospital B, *E. coli* isolates were referred to the Research Laboratory for Infectious Disease, NIHRD, to perform an ESBL-producing *E. coli* confirmatory test.

### Table 1

| Variable                  | Human sector | Animal sector/food chain | Environment sector |
|--------------------------|--------------|--------------------------|--------------------|
| Sample                   | Pregnant women | Bloodstream infection patient | broiler cecum |
| Number of samples        | 100           | 116                      | 240               |
| Sampling sites           | 1 Primary Health Care (PHC) Facility | 2 hospitals | 6 markets/slaughterhouses |
| Sampling time            | 10 months     | 14 months                | 10 months         |
| Epidemiology data        | yes           | yes                      | yes               |
| Laboratory               | NIHRD*        | Hospital Lab and NIHRD** | DIC***            |
| Primary culture          | MacConkey and MacConkey + CTX Bactec | MacConkey + CTX TBX and TBX + CTX |
| *E. coli* identification | indole test   | Vitek-2                  | indole test       |
| ESBL identification +    | Vitek-2       | DDST***                  | DDST****          |

*The Research Laboratory for Infectious Diseases, NIHRD, Jakarta **Disease Investigation Center Subang, West Java, ***the Centre for Research and Development of Environment Quality Laboratory, Banten, ****Double Disk Sinergy Test.

#### 2.2. Sample collection and examination

The sample collection and laboratory examination methods of this study referred to the guidelines of the Tricycle Project. Modifications were made to incorporate the Epi-X method in the epidemiology and to adapt to field conditions. For patients with bloodstream infections, the identification of *E. coli* producing ESBL was carried out in a hospital laboratory according to the procedures established in each hospital (Table 1). A total of 14,682 samples of blood cultures from two hospitals (A and B) were examined from October 2018 to December 2019. The identification of ESBL-producing *E. coli* in Hospital A was carried out using Vitek-2 (bioMérieux) in the hospital laboratory, while in Hospital B, *E. coli* isolates were referred to the Research Laboratory for Infectious Disease, NIHRD, to perform an ESBL-producing *E. coli* confirmatory test.
using Vitek-2 (bioMérieux). In the community setting, recruitment of healthy pregnant women was based on voluntary participation (convenience sample). One hundred rectal swabs were collected from pregnant women who received ANC in a PHC facility. The rectal swabs were collected by trained midwives, placed in the Cary-Blair medium and transported to the Research Laboratory for Infectious Disease, NIHRD, within 24 h after collection for ESBL-producing *E. coli* identification.

For the animal sector, a total of 240 broiler carcasses were collected from six markets/slaughterhouses. The cecal samples were harvested aseptically on site and placed in a sterile container at 2–4 °C. Samples were transported to the Diseases Investigation Centre (DIC) Subang, Ministry of Agriculture within 24 h after collection. For the environment sector, water samples were collected from January to October 2019 that cover both the dry and wet seasons. One hundred milliliters of water was collected from each site every month and stored in a sterile bottle at 4 °C. The samples were transported to the Centre for Research and Development of Environment Quality Laboratory within 24 h of collection for ESBL-producing *E. coli* identification. A total of 119 out of 120 water samples were examined in this study; one sample was excluded due to a technical sampling error.

The questionnaire was used to obtain epidemiological data from respondents in the human and animal sectors, and the characteristics of environmental samples were collected using a sample collection form. The sample characteristics, including water temperature, pH, salinity, season and environmental conditions, were measured and observed. All ESBL-producing *E. coli* isolates were stored in tryptase soy broth (TSB) + 15% sterile glycerol at −80 °C for further analysis by WP4 (whole genome sequencing) in NIHRD. Sequencing and data analysis will be conducted in the Research Laboratory for Infectious Disease, NIHRD. Unfortunately, at the time this article was written, sequencing and bioinformatics analysis had to be postponed due to unforeseen challenges.

2.3. Statistical analysis

Simple analysis was performed by using SPSS version 15.0 and Microsoft Excel 2010.

3. Results

3.1. Characteristic of subjects

Table 2 presents the characteristics of pregnant women, patients with bloodstream infections, broiler sellers or owners of the slaughterhouses, and environmental samples. The demographics of pregnant women covered all age groups, numbers of pregnancies, and gestational ages. Bivariate analysis revealed that there was no correlation between the characteristics of pregnant women shown in Table 2 and ESBL-producing *E. coli* colonization (data not shown). On the characteristic of subjects with bloodstream infections, some of those infected by ESBL-producing *E. coli* were under 5 years old. This age group was predicted as the first or the second highest prevalence based on the range of age. Almost half of the subjects (48.0%) were diagnosed with malignancies, and more than half of the subjects were receiving beta lactam antibiotics when the data were collected. In the Tricycle Project, it is difficult to statistically determine the correlation of the characteristics of the patient with bloodstream infections and the proportion of ESBL-producing *E. coli*, since the inclusion criteria of the subject was a patient with bloodstream infections caused by ESBL-producing *E. coli*. Data on patient characteristics with non-ESBL-producing *E. coli* infection were not available.

3.2. ESBL-producing *E. coli* in the human sector

ESBL-producing *E. coli* was isolated from 40 (40%) of 100 healthy pregnant women (Table 3), which meant that at least four out of ten pregnant women were carriers of ESBL-producing *E. coli*. *Escherichia coli* was also the frequent cause of bloodstream infections, which accounted for 118 (10.4%) of 1135 bacteria isolated from blood cultures in...
Hospital B and 83 (15.8%) of 525 bacteria isolated from blood cultures in Hospital A. A total of 63 (53.4%) isolates of 118 *E. coli* were identified as ESBL-producing *E. coli* in Hospital B, while 53 (63.9%) isolates of 83 *E. coli* were ESBL-producing *E. coli* in Hospital A. In this study, ESBL-producing *E. coli* were isolated from 63 (0.6%) of 9812 total blood cultures performed in Hospital B, while the bacteria were isolated from 53 (1.1%) of 4870 blood cultures performed in Hospital A. There was slight difference in these proportions between Hospitals A and B. On average, ESBL-producing *E. coli* was found in 0.8% (116/14,682) of total blood cultures, 7.0% (116/1660) of total positive bacterial cultures, and average, ESBL-producing *E. coli* was found more frequently in the animal (food chain) sector (Table 4) than in healthy human samples (Table 3), with 3.3% of total *E. coli* isolates identified as ESBL-producing *E. coli* in the animal (food chain) sector (Table 4) than in healthy human samples (Table 3), with proportions of 67.1% vs. 40%. The proportion range did not seem to be significantly different (62.5%–77.5%) between the sampling locations. However, it clearly varied between sample collection events, ranging from 8.3% (XIII) to 100% (I, IV, VI, and VII). The reason behind the difference remains unclear and need to be investigated further. Table 4 shows that 161 (84.3%) out of 191 suspected colonies on MacConkey agar supplemented with 0.4% cefotaxime medium were confirmed as ESBL-producing *E. coli*.

### 3.3. ESBL-producing *E. coli* in the animal (food chain) sector

ESBL-producing *E. coli* was found more frequently in the animal (food chain) sector (Table 4) than in healthy human samples (Table 3), with proportions of 67.1% vs. 40%. The proportion range did not seem to be significantly different (62.5%–77.5%) between the sampling locations. However, it clearly varied between sample collection events, ranging from 8.3% (XIII) to 100% (I, IV, VI, and VII). The reason behind the difference remains unclear and need to be investigated further. Table 4 shows that 161 (84.3%) out of 191 suspected colonies on MacConkey agar supplemented with 0.4% cefotaxime medium were confirmed as ESBL-producing *E. coli*.

### 3.4. ESBL-producing *E. coli* in the environment sector

All samples collected from upstream through downstream contained ESBL-producing *E. coli* with varying concentrations and ratios, log 2.8–7.3 CFU/100 mL and 4.2–30.2% of total *E. coli* (Table 5). An average pH under 6.5 was only found in Ciplak (market wastewater), while an average salinity above 2.0 ppm was only found in Cilincing, which is located near the sea. However, in both locations, the *E. coli* and ESBL-producing *E. coli* concentrations were not lower than those in other locations according to the same criteria. The highest concentration of ESBL-producing *E. coli* was found in Ciplak market wastewater (log 8.5 and log 7.3 CFU/100 mL), while the highest ratio of ESBL-producing *E. coli* to total *E. coli* was found in Rw. Keeping slaughterhouse wastewater (30.2%). The lowest ratio of ESBL-producing *E. coli* was found in Molek surface water (4.2%), although the *E. coli* and ESBL-producing *E. coli* concentrations at this site were the highest among the three up/midstream sites. On the other hand, the East Flood Canal (BKT) had lower *E. coli* and ESBL-producing *E. coli* concentrations among the three downstream sites.

Salinity was not examined at sample collection events I, II, and IX because it is only an additional indicator in the Tricycle Project. The averages of pH, salinity, temperature, *E. coli* and ESBL-producing *E. coli* concentrations were not significantly different among sample collection events. However, the ratio of proportion of ESBL-producing *E. coli* clearly differed between the dry season and rainy season, 9.5–16.5% vs. 5.9–30.6%. This parameter showed a much wider range in the rainy season. In addition, Indonesia has only two seasons, dry and rainy.

### 4. Discussion

Geographic information systems (GISs) and analyses based on GISs have become widespread and well accepted approaches to understand the interaction between humans, animals, and the environment. In this study, GIS was used to identify sampling sites across sectors (human, animal, and environment) to determine the correlation and interaction of AMR bacteria in three sectors. The human sampling sites were selected based on the strict requirement stated in the protocols, especially the minimum number of blood cultures in the hospital in a year. The sampling sites for animals and the environment were chosen based on the locations where humans and animals are supposed to interact and induce the transmission of ESBL-producing *E. coli*. An additional three environmental sampling sites (up/midstream) before the markets/slaughterhouses and three sites after them (downstream) were used to assess the effect of activities in the markets/slaughterhouses on the contamination with ESBL-producing *E. coli* in the environment. The analyses of upstream and midstream samples were combined into one variable (up/midstream) because the distance between both sites is not too far geographically and the upstream sample collection sites are not amidst water springs but located near residences (Fig. 1).

In this study, the ratio of ESBL-producing *E. coli* to total *E. coli* was high in humans, both in healthy and sick people. In healthy people, ESBL-producing *E. coli* was found in 40.0% of pregnant women (Table 3), which is approximately three times as high as the results from a similar research conducted at almost the same time but in different provinces in Indonesia [12]. The differences in the prevalence of ESBL-producing *E. coli* could be influenced by geographical factors and the method. Therefore, it is important to conduct AMR surveillance with the standardized method while adhering to the guidelines defined in the Tricycle Project. From a global perspective, the prevalence of AMR in high-income countries is generally lower than that in low-middle-income countries. A study in Switzerland described that ESBL-producing *E. coli* was found only in 2.7% of pregnant women; in contrast, ESBL-producing *E. coli* in pregnant women was high in several African countries, especially Cameroon (57%) [13]. In low- and middle-income countries, the poor hygiene and sanitation as the primary route of infection increased antimicrobial usage and facilitated the rise of AMR prevalence [14]. Poor hygiene practice in healthcare setting and food chain allow the resistance bacteria transmission [15]. This is thought to be the cause of this variability between contexts.

Table 3 also shows that *E. coli* is the main pathogen that causes bloodstream infections, and the majority of them were ESBL-producing *E. coli*. These findings are in line with the GLASS report in 2019 in which *E. coli* caused most bloodstream infections (26.5% of a total of six bacteria), and the prevalence of ESBL-producing *E. coli* in bloodstream infections reached >50% out of total *E. coli* infections. If the AMR control program does not consider these data seriously, Indonesia will face...
conditions like several countries with high prevalence of ESBL-producing E. coli, i.e., India (72.4–87.3%), Nigeria (70.5–92.3%), and Bangladesh (63.2–68.4%). In contrast, high-income countries reported generally low prevalence of ESBL-producing E. coli as the pathogen causing bloodstream infections, i.e., Netherlands (7.3%), Switzerland (7.3–10.2%), Japan (9.0–20.1%), and Germany (12.2%) [5, 16] similar to the prevalence of ESBL-producing E. coli in pregnant women in those countries. This fact is a challenge to Indonesia to make serious efforts to control AMR problems. Indonesia may refer to its neighboring countries, Malaysia and the Philippines that have similar population characteristics and geographic condition but ESBL E. coli prevalence in bloodstream infections is much lower than that in Indonesia. In the 2019 GLASS report, Malaysia and the Philippines reported ESBL-producing E. coli prevalence of 18.5–27.3% and 26.2–40.7%, respectively.

Based on patient characteristics, most ESBL-producing E. coli isolates were from patients with malignancies receiving third-generation cephalosporine therapy (Table 2). E. coli infection is common in patients with malignancies due to suppressed immune status [17]. Bedalsaz-Minooz et al. showed that bloodstream infections in cancer patient were mostly caused by secondary infections localized in the urinary tract and abdominal sources. Another main source of bloodstream infection is the central venous line [18]. Based on these facts, infection prevention controls such as personal hygiene, restriction of infection sources, and implementation of infection prevention principles during the placement and maintenance of catheter devices should be strictly observed in cancer patients. Additionally, the patient characteristics also indicate that ESBL-producing E. coli infection correlates with 3rd generation cephalosporine therapy. This shows the risk of AMR caused by the selection pressure mechanism. It is important to control 3rd-generation cephalosporine usage and implement infection prevention control in hospitals [15] as a part of the implementation of the antimicrobial stewardship program.

The high proportion of ESBL-producing E. coli in food chains (Table 4) can be due to many factors, including the use of antibiotics in livestock and contamination of livestock feed with ESBL-producing E. coli [19, 20]. There were some previous reports on ESBL-producing E. coli in broilers, including those from Indonesia. Wibisono et al. reported that ESBL-producing E. coli was found in 28.75% of broiler cloacal swabs in Blitar, East Java, Indonesia, with a significant variation in the positive rates between sample collection sites (8–100%) [21]. Similar conditions were reported in India, where the detection frequency of ESBL-producing E. coli varied widely between states [22, 23]. The significant difference in the prevalence of ESBL-producing E. coli between regions could be due to the implementation of regulation. Many countries, including Indonesia, have regulations that prohibit the use of antimicrobials as growth promoters [24]. However, the enforcement of this regulation varies in each country or region [25].

The proportion of samples containing ESBL-producing E. coli varied between sampling events (Table 4). This is one of the advantages of the Tricycle Project, which collects serial samples so that sampling bias can be minimized. It is interesting to note that the high percentage of ESBL-producing E. coli in the food chain does not always correlate with the frequency of ESBL-producing E. coli in bloodstream infections. Similar observations were made in the Netherlands in some studies [5, 26, 27]. Although high percentages of ESBL-producing E. coli were isolated from broilers, the prevalence of ESBL-producing E. coli in blood cultures in the Netherlands was low, as described in the 2019 GLASS report. This is a good paradigm that Indonesia and other countries need to emulate, where we still face obstacles in controlling AMR in the animal sector but have the capability to control AMR in the human sector.

A good correlation was observed between the percentages of colonies growing on MacConkey medium supplemented with 0.4% cefotaxime and that of ESBL-producing E. coli. This indicated that the medium could be used for screening or presumptively identifying ESBL-producing E. coli in food chain samples. Previous study reported MacConkey medium supplemented with 0.4% Cefotaxime reliable to screen ESBL-producing E.coli in water and fecal sampel. The study reported MacConkey supplemented with 0.4% Cefotaxime can inhibit the growth of AmpC-producing E.coli that is not inhibited on MacConkey supplemented with 0.2% Cefotaxime. The use of this medium will make
laboratory easier to isolate the ESBL-producing E.coli and inexpensive. However, the quality of MacConkey medium varied by manufacturer, therefore, it is important to use the same manufactured to have the standardized quality [28].

The concentration of ESBL-producing E. coli in the environment sector found in this study was also very high (Table 5) compared to other countries, including the Netherlands [29], Switzerland [30], and Ecuador [31]. The high concentration of ESBL-producing E. coli in the environment sector might lead to the high concentrations of ESBL-producing E. coli in the animal and human sectors. River water is used by farmers on riverbanks to water vegetables and fruits, while some vegetables and fruits will be consumed by humans without cooking processes. The river water is also used to feed livestock [32,33]. Moreover, some people dispose of human and animal waste into the river and use manure to fertilize vegetable and fruit crops. The East Flood Canal (Banjir Kanal Timur/BKT) had the lowest E. coli and ESBL-producing E. coli concentrations among the three downstream sites. It is presumed that the BKT, as an artificial canal, does not receive any wastewater flow from markets or slaughterhouses directly (Fig. 1). Therefore, it is important to include environmental factors in AMR surveillance and tackle waste management to break the transmission cycle of AMR bacteria. The environment facilitates the transmission of AMR bacteria because bacteria, especially E. coli, can survive in the environment for months or even years. AMR due to selection pressure can also occur in the environment [15,34].

There are several factors that affect the survival and growth of E. coli, including physical, chemical, and biological factors, such as temperature, pH, oxygen, phosphates, ammonia, lactate, salt, and chlorophyll [35–38]. However, in this study, temperature, pH and salinity did not appear to have an effect on E. coli concentrations (Table 5). In this study, temperature, pH and salinity did not significantly vary among locations, and no extreme values were found. Previous studies have shown that E. coli might tolerate temperature changes, acidic conditions and an increase in sodium concentration [38,39].

AMR surveillance with the “One Health” approach is a good strategy to control AMR bacteria transmission, especially ESBL-producing E. coli. A previous study has proven the relationship between ESBL-producing E. coli in humans and other sectors [40]. As a model for implementing AMR surveillance with a “One Health” approach in Indonesia, the Tricycle Project is highly meaningful. Nevertheless, we encountered several obstacles during its implementation. AMR control has not yet become a program priority at the Ministry of Environment and Forestry. AMR is a new issue in this ministry. This will be a challenge when surveillance has to be implemented on regular basis and the budget has to be allocated by ministries. The limited human resources in the hospital and the number of questions in the questionnaire were the reasons why the questionnaire was not fully completed. Several antibiotics used in ESBL confirmatory tests are not available in Indonesia, and not all laboratories that participated in this study have the facility to perform comprehensive laboratory examinations according to the project guidelines. Furthermore, the bureaucratic complexity of interministerial coordination poses an additional layer of challenges.

This study has several limitations in the sampling method, including the recruitment process and number of samples. Basically, the study was not designed as a research study but for surveillance purposes. There were some limitations in the available data for statistical analysis. The recruitment of pregnant women did not use a random sampling method to avoid bias, and the number of statistical tests did not well distribute. The sample size was not defined by statistical methods. However, some of the limitations discussed above have been minimized in the updated Tricycle Project guidelines. To collect additional information on the burden of AMR prevalence throughout the year, the number of sampling events for environmental samples was expected to be increased to 8–12 times a year according to the updated guidelines. In the present study, environmental sampling was carried out ten times according to the Epi-X protocol.

We propose several recommendations to minimize obstacles and limitations. Since this study was designed as a surveillance, it is important to ensure the distribution of pregnant women to represent local demographics of human samples, and the collection of food chain and environmental samples need to be evenly distributed each month. To ensure that the questionnaire will be filled out completely, it is necessary to consider simplifying the questionnaire, in line with the updated guideline. Limitations related to the facility include those under the Ministry of Health and provincial government that can be expected to participate in the future. Regarding the availability of antibiotics, the confirmatory test in the next project will be modified based on the availability of the antibiotic disks in Indonesia. As an alternative to cefotaxime-clavulanic acid, which is not available in Indonesia, the study will use amoxicillin-clavulanic acid.

We also propose policy recommendations to AMR program stakeholders in the three sectors, human, animal, and environment. We recommend the continuation to the next working package and expansion of this study to other provinces to determine the AMR burden to establish the baseline for planning AMR control strategies in Indonesia. This project also needs to be developed as ongoing surveillance to update data and evaluate the progress of AMR programs and interventions by adopting this surveillance in AMR control program activities, especially to reduce the prevalence of ESBL-producing E. coli in all sectors as an indicator. By referring to the results of this study, a comprehensive strategy should be implemented to control AMR in Indonesia, such as improving AMR regulation and policy, especially in antimicrobial usage, strengthening the AMR surveillance system, encouraging the implementation of antimicrobial stewardship programs, infection prevention control programs, and animal husbandry, and improving research that aims to find interventions as alternatives to antibiotics.

5. Conclusion

The Tricycle Project (pilot) in Indonesia identified that the proportion of ESBL-producing E. coli was very high in all types of samples, and several challenges and obstacles were found during implementation in the field. We have made recommendations to AMR program stakeholders in the three sectors, humans, animals, and the environment, and proposals to minimize obstacles in the future.

Credit author statement

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**Declaration of Competing Interest**

The authors declare that they have no conflicts of interest.

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