The effect of environmental factors on the abundance of cefotaxime-resistant *Escherichia coli* in Sunter River

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**Abstract.** The water quality of the Sunter River in Jakarta was classified as heavily polluted due to activities around the river, both domestic and non-domestic. As one of the environmental parameters for water quality, the presence of *Escherichia coli* (*E. coli*) is normally found in any natural environment, and under certain conditions it can become resistant to antimicrobials due to genetic mutations. The mutated *E. coli* produces Extended Spectrum Beta-Lactamase (ESBL) enzymes and has a higher survival ability in antibiotic-contaminated river water, thus potentially endangering public health. This study was aimed to evaluate the effect of environmental factors on the abundance of ESBL producing *E. coli* and their resistance to antibiotic cefotaxime. Sampling was conducted in six locations representing the upstreams and downstreams of Sunter River, following the Global Surveillance guidelines. *E. coli* strains were isolated using Tryptone Bile X-glucuronide (TBX) agar medium (with and without the addition of cefotaxime 4μg/ml) and the antibiotic sensitivity test of ESBL *E. coli* was conducted by performing a double-disk test. The results showed that the highest average abundance of ESBL *E. coli* was found in the sample taken from Sindang Station (904.24 x 10⁴ CFU / 100 mL) and the lowest was from Sunter Station (1.58 x 10⁴ CFU / 100 mL). The results of the Bivariate Pearson correlation analysis showed that temperature, pH, and salinity were negatively correlated with the abundance of ESBL-producing *E. coli* bacteria.

1. **Introduction**

According to the current provisions of the World Health Organization (WHO) and American Public Health Association (APHA), water quality is determined by the presence and number of bacteria in it. Several types of bacteria live in water, such as coliform bacteria and *E. coli*. The presence of coliform bacteria in water can be used as a determinant of whether the water is suitable for certain purposes such as drinking water, fisheries, animal husbandry, agriculture, and others [1].

*Escherichia* is a microorganism that was originally known to live in the human body as a contaminant and isolated from human feces, then spread not only to humans but also to ecosystems [2]. According to [3][4], the abundance of *E. coli* in water can be used as an indicator of the presence of pathogenic bacteria. *E.coli* is found in soil and water due to fecal contamination [5][6][7]. In addition, *E. coli* is found in air and dust, and is particularly used as an indicator of antimicrobial resistance [8][9]. *E. coli* can become resistant to antimicrobials due to gene mutations, such as the...
gene encoding beta-lactamase, an enzyme that specifically breaks down the chemical structure of the beta-lactam ring of certain types of antimicrobials, so that bacteria can become resistant to this drug [10][11]. Beta-lactamases enzymes are called Extended Spectrum Beta-Lactamases (ESBL), listed as third-generation broad-spectrum cephalosporins (eg., cefotaxime, ceftriaxone, and ceftazidime) and monobactams (eg., aztreonam). ESBL will cause resistance to penicillin antibiotics, cephalosporins, and aztreonam, as well as to other classes of antibiotics, including aminoglycosides, trimethoprim-sulpha metoxazole, and quinolones. Several studies [12][13][14] found ESBL-producing bacteria in hospital treatment rooms, and ESBL-producing E. coli in environmental samples that were resistant to 100% penicillin, 100% amoxicillin, 70% streptomycin, 60% trimethoprim-sulfamethoxazole, and 30% tetracycline, and has the potential to spread resistance genes to other bacteria.

Environmental condition is one key factor of gene mutation. A study by [15] shows the important components that cause the transmission of resistant bacteria and the emergence of resistant bacteria through the evolutionary and ecological processes of the clinical nature of resistance genes due to the pressure of environmental selection to survive. Environmental stress can also occur in the gene for E. coli resistance through the diffusion of antibiotics in the agricultural food system [16].

The misuse and overuse of antibiotics in human health, animal husbandry, and agriculture, together with poor management of wastes originating from those activities, causing antibiotics contamination on rivers which eventually becoming a source of antibiotics-resistant microbes infection to humans and animals [17][18][19].

Sunter River is one of thirteen rivers that flow in Jakarta. The upstream of the Sunter River is located in the Cimpaeum sub-district, Depok City, passing through the Cipinang Melayu sub-district, Makassar district which is the eastern part of the Daerah Khusus Ibukota (DKI) Jakarta province, and the downstream is located in Tanjung Priok, East Jakarta. The Sunter River area is densely populated, with the dominant type of pollutant entering the river in the form of organic waste originating from settlements, traditional markets, and chicken slaughterhouses [20]. In addition, the water quality of the Sunter River is also largely determined by agricultural activities that produce and dispose of waste into the river. Pollution due to community activities around the river indirectly affects the concentration of E. coli and ESBL-producing E. coli. Meanwhile, according to the Dinas Lingkungan Hidup (DLH) Jakarta report in 2017, the status of water quality in Sunter River was classified as heavily polluted as stipulated in the Environmental Minisitral Regulation No. 115 of 2003 and exceeding the capacity limit as regulated in the Government Regulation No. 82 of 2001. Parameters that must be measured to determine water quality include temperature, turbidity, color, electrical conductivity (EL), total dissolved solids (TDS), taste, and smell [21]. This research was conducted to determine the effect of environmental parameters such as temperature, pH and salinity on the abundance of E. coli and ESBL-producing E. coli in the Sunter River.

2. Method
2.1. Study area and sampling stations
This study was conducted in Sunter river which has watershed area of 1,080 ha with 37.25 km length, 73,18 km² flow area, with surface width of 10 m, base width of 3 m and depth of 1.40 m in average, minimum discharge of 0.86 m³/s and maximum discharge of 5.84 m³/s.
Figure 1. The sampling stations distribution in DKI Jakarta province.

The samples were collected from six sampling stations (Table 1) chosen using purposive sampling method to represent the upstream area (Molek, Sunter, Kranggan) and the downstream area (Sindang, Cilincing, and BKT). The sampling was carried out for 10 consecutive months from December 2018 to October 2019 and was performed following the Global Surveillance guidelines.

Table 1. GPS coordinates of the sampling stations.

| Sampling Station | GPS Coordinate |
|-----------------|----------------|
| Molek           | 06'17'04.0" S  |
|                 | 106'54'31.4" E|
| Sunter          | 06'19'04.0" S  |
|                 | 106'55'17.2" E|
| Kranggan        | 06'21'56.5" S  |
|                 | 106'54'51.2" E|
| Sindang         | 06'06'48.3" S  |
|                 | 106'53'48.7" E|
| Cilincing       | 06'06'55.4" S  |
|                 | 106'56'26.0" E|
| BKT             | 06'06'38.1" S  |
|                 | 106'58'08.8" E|

Surface water were sampled from the middle of the river using Grab sampler for several times each station and then were homogenized in a bucket, stored in 250 ml sterile glass, and kept in a cool box with a temperature of 4°C for later examination in the laboratory within 24 hours. Field parameter, such as pH, salinity, and temperature were measured in each station using calibrated tools.

2.2. *Escherichia coli* isolation

Water samples from each sampling station were filtered using membrane filter with 0.45 μm mesh size. One ml of the filtered water samples was swabed to Tryptone Bile X-glucuronide (TBX) agar media prepared in petri dishes, with and without the supplementation of cefotaxime 4μg/ml. After incubation at the temperature of 35°C for 24 hours, macroscopic observation was done on each of the petri dish to select five presumptive colonies of *E. coli* and ESBL-producing *E. coli* randomly. Colonies that were round, blue-green, and surrounded by a cloudy zone were suspected as *E. coli* and/or ESBL-producing *E. coli.*
2.3. Biochemical identification
The identification of *E. coli* was carried out conventionally with biochemical method using Kovac’s reagent to identify the ability of bacteria to produce indole from tryptophan [22]. *E. coli* cells, as much as one loop of inoculation needle, from each selected colony was inoculated aseptically in culture tubes and incubated at 37°C for 24 hours. The positive indole test were indicated by the presence of a red ring due to the reaction of indole with aldehydes [22, 23].

2.4. Antibiotic sensitivity test
All isolates confirmed as *E. coli* were further tested for confirmation of the ESBL-producing *E. coli* using double disk test [24]. The isolate suspension was prepared with a turbidity equivalent to 0.5 McFarland, and then spread on Mueller–Hinton (MH) agar medium using scatter rods. Four antibiotics disc, namely clavulanacid ceftazidime (30 μg), ceftazidime (30 μg), clavulanacid cefotaxime (30 μg), and cefotaxime (30 μg) were placed in the petri dishes in pairs. After incubation at 37°C for 24, the inhibitory zone diameter (IZD) was measured as the clear zone around each disk using a caliper.

The presence of ESBL-producing *E. coli* was expressed by the difference of the IZD between clavulanacid ceftazidime (30 μg) and ceftazidime (30 μg) or between clavulanacid cefotaxime (30 μg) and cefotaxime (30 μg) of more than 0.5 mm [14].

2.5. Data analysis
The concentration of inoculated *E. coli* on TBX agar medium, with and without the addition of cefotaxime 4μg/ml, was calculated using the formula:

\[ C = \frac{Z}{V_{\text{tot}}} x V_{\text{rep}} x b/a \]  

where:
- \( C \) : Inoculated *E. coli* concentration
- \( Z \) : Total number of countable colonies on plates with <200 colonies (CFU)
- \( V_{\text{tot}} \) : Total volume of water sample inoculated on the plates
- \( V_{\text{rep}} \) : Reporting volume (100 ml)
- \( a \) : Number of colonies taken for ESBL-producing *E. coli* confirmation
- \( b \) : Number of colonies confirmed as ESBL-producing *E. coli*

The percentage of ESBL-producing *E. coli* was calculated relatively to the total number of *E. coli*. To determine the relationship between *E. coli* abundance and water quality parameters, the Bivariate Pearson correlation test was performed. The analysis was carried out using SPSS ver.19.00. The interpretation of the strength of the relationship between two variables was made with the following criteria: 0.00 – 0.20 “very weak or very low correlation”; 0.20 – 0.40 “weak or low correlation”; 0.40 – 0.70 “medium correlation”; 0.70 – 0.90 “strong or high correlation”; 0.90 – 1.00 “very strong or very high correlation” [25].

3. Results and discussion
3.1. *Escherichia coli* colonies
Figure 2 shows the form of *E. coli* colonies growing on TBX agar media (a), and the antibiotic sensitivity test of *E. coli* colonies on MH agar media as chromogenic media containing bile salts (b). The high content of bile salts in the media can inhibit the growth of Gram-positive bacteria, and with cefotaxime supplementation it can be an indicator of the presence of ESBL-producing *E. coli* [26]. *E. coli* colonies grown on TBX agar media with cefotaxime supplementation were used for the confirmation of ESBL-producing *E. coli* by performing the indole test and double disk test.
Figure 2. a) Colonies of E. coli grown on TBX agar medium with cefotaxime supplementation; b) the confirmation of ESBL-producing E. coli by double disk test.

The concentration of E. coli in water samples taken from six sampling stations along the Sunter river are shown in Figure 3. The highest average concentration of E. coli was found in water sample taken from Sindang (8,375.35 x 10⁴ CFU / 100 mL) and the smallest average concentration was found in the water sample taken from BKT station (13,28 x 10⁴ CFU / 100 mL).

![Figure 3](image)

Figure 3. The concentration of E. coli (10⁴ CFU/100 mL) sampled from Sunter River.

The high concentration of E. coli at this station is probably caused by water pollution from surrounding activities such as markets and residentialities more than other stations, which can be seen from several waterways that enter the river, and BKT is the downstream of the river with the largest
river width compared to other sampling stations, where all pollutants congregate [27]. According to [20], the large volume of water in the Sunter river can be utilized for urban businesses and the hydroelectric power industry.

![Figure 4](image)

**Figure 4.** The concentration of ESBL-producing *E. coli* (10^4 CFU/100 mL) sampled from Sunter River.

ESBL-producing *E. coli* was found in water sample taken from Sindang 904.24 x 10^4 CFU / 100 mL). Meanwhile, the smallest average concentration of ESBL-producing *E. coli* was found in the water sample taken from Sunter station (1.58 x 10^4 CFU / 100mL). The water volume in Sunter station was considerably low, yet water currents was present, the riverbed was not muddy, and pollution from the surrounding community settlements was inconsequential.

According to [28], one of the antibiotic contaminants in rivers and the environment from industrial waste, untreated hospital effluent, and untreated municipal wastewater is likely to cause higher concentrations of ESBL-producing *E. coli* and will gradually lead to surface water contamination.

As shown in Figure 5, ESBL-producing *E. coli* was found in water samples from all sampling stations. The presence of ESBL-producing *E. coli* is found in many other rivers, such as the Cikapundung river [27], the Danube river [28], lakes and rivers in northern China [29] and the Asir river in Turkey [30].

![Figure 5](image)

**Figure 5.** Percentage of ESBL-producing *E. coli*. 
ESBL-producing *E. coli* are resistant to antimicrobials due to the gene mutation which resulted in the capability to encode beta-lactamase [14]. Beta-lactamase is an enzyme that specifically breaks down the chemical structure of the beta-lactam ring of antimicrobial [17]. Several different bacterial genera can acquire the gene encoding ESBL, which clinically is of particular concern, due to the severity of the disease and the difficulty of treatment, including ESBL-producing *E. coli* and *Klebsiella spp* [31]. These bacteria can respond to selective pressure and quickly adapt to new environments by acquiring new genetic traits as a result of mutations, which occur relatively slowly but mostly resulting in pathogenic traits.

### 3.2. Environmental factors

Measurement of temperature, pH and salinity of the Sunter river water was recorded during sampling. In general, the measurement results showed that the environmental condition in the six stations were comparable and fitted the water quality classification according to the Government Regulation No. 22 Year 2021.

**Table 2. The results obtained on the measurement of chemical parameters of Sunter River.**

| Sampling Station | Temperature (Min. – Max.) | pH (Min. – Max.) | Salinity (Min. – Max.) |
|------------------|--------------------------|-----------------|-----------------------|
| Molek            | 30.08 (27.5 - 31.6)       | 7.28 (6.8 - 7.72) | 0.3 (0.1 - 0.5)       |
| Sunter           | 29.5 (27.6 - 31.2)        | 7.10 (6.75 - 7.58) | 0.2 (0.1 - 0.6)       |
| Krangganan       | 29.10 (25.70 - 31.10)     | 7.23 (6.94 - 7.74) | 0.1 (0.1 - 0.2)       |
| Sindang          | 30.61 (27.5 - 32.5)       | 7.20 (6.74 - 7.54) | 1.3 (0.3 - 2.4)       |
| Cilincning       | 31.35 (26.8 - 33.9)       | 7.17 (6.7 - 7.55)  | 2.6 (0.3 - 5.6)       |
| BKT              | 31.84 (28.9 - 33.7)       | 7.73 (7.07 - 8.81) | 1.0 (0.1 - 5.6)       |

Note: The quality standard for water quality according to the Government Regulation No. 22 Year 2021, Appendix VI: *water temperature deviation to the surrounding temperature is maximum 3°C; **water pH is between 6 to 9.*

*E. coli* bacteria can grow at the temperature of 7 to 44°C and grow optimally at 37°C [32][33]. In addition, *E. coli* can live in humid places, are relatively sensitive to heat, and will die by pasteurization [34]. The effect of heat will induce protein misfolding and aggregation in *E. coli* cells including in its membranes, cytoplasm, ribosomes, and DNA, thus in order to encounter the overheating, *E. coli* has the mechanism to change its genes for the expression of key proteins required in the outer membrane formation, and to overcome the osmotic pressure of the compatible solute [35]. A study reported that *E. coli* survived in the temperature up to 60°C for 15 minutes and 55°C for 60 minutes [36].

Furthermore, *E. coli* can grow in the environment with pH range between 4 to 9, and optimally with pH of 7 to 7.5 [37]. [38] explained that the decrease of the pH value in water occurs due to the formation of carbonic acid (H₂CO₃) due to the dissolved carbon dioxide (CO₂) in the water, and the release of H⁺ ions. Furthermore, [39] stated that hydroxide ions (OH⁻) can be produced in aqueous solutions under acidic conditions. A study by [40] showed that *E. coli* has higher survival rate under acidity condition with dissolved oxygen.

Meanwhile, the optimum salinity for the growth of coliform bacteria is not greater than 85‰ [41]. [32] stated that the optimal growth of *E. coli* and *Staphylococcus aureus* occurred at a concentration of 0% (w/v) NaCl. However, increasing the NaCl concentration above 0% to 1.0 and 3.0% decreased the growth of *E. coli* and *S. aureus* at 37°C.
Bivariate Pearson's correlation analysis done in this study were interpreted in association with the Product Moment (rxy) correlation [42]. As shown in Table 4, the highest correlation was obtained from pH in both E. coli and ESBL-producing E. coli, while the lowest correlation was from the salinity. However, the water temperature, pH, and salinity values recorded in this study have very weakly correlation to the abundance of E. coli.

**Table 3.** Bivariate Pearson's correlation analysis on the abundance of E. coli.

|            | Water Temperature | pH    | Salinity |
|------------|-------------------|-------|----------|
| E. coli    | -0,099            | -0,191| -0,015   |
| ESBL-producing E. coli | -0,100 | -0,196| -0,001   |

Note: (-) negative correlation (opposite), (+) positive correlation (unidirectional)

The water temperature has a negative correlation with the abundance of E. coli and ESBL-producing E. coli, which means that the higher the temperature, the lower the abundance of E. coli and ESBL-producing E. coli. E. coli can grow well at temperatures between 8° to 46°C with the optimum temperature below 37°C [43]. Water temperature that exceed the normal limits indicates the high level of dissolved chemicals and decomposition process of organic matter is being carried out by microorganisms [44].

Moreover, the water pH has negative correlation as well with the abundance of E. coli and ESBL-producing E. coli, meaning that the abundance of E. coli and ESBL-producing E. coli will decrease with the increase of water pH. According to [5], acidity is a limiting factor for the growth of E. coli [45][46].

Water salinity in this study also had a negative correlation value to the abundance of E. coli and ESBL-producing E. coli. As explained by [47], the growth of E. coli decrease due to the increase of NaCl, and causing sudden change of cell metabolism at the NaCl concentration threshold.

4. Conclusion
From the 6 sampling stations, the highest abundance of E. coli and ESBL-producing E. coli was found in Sindang station, influenced by water pollution from surrounding activities. The abundance of cefotaxime-resistant E. coli mostly was affected by pH, compared to temperature and salinity. Although E. coli bacteria have undergone gene mutations to become antibiotic-resistant, yet their abundance is influenced by the environmental factors.

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8
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