Phenotypic analysis of antibiotic resistant *E. coli* recovered from urban aquatic environment in Banda Aceh, Indonesia

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Abstract. Of aquatic environment, antibiotic resistant bacteria, including total coliforms and *E. coli* disseminate and emerge at an alarming rate. The study aims to determine enumerate, isolate, and determine their antibiotic resistance and compare between those which were recovered from residentials and home industries in Banda Aceh and its surrounding area. The bacterial density and antibiotic susceptibility of total coliforms and *E. coli* were determined using Standard Total Coliform Multiple-Tube (MPN) Fermentation method and the disk diffusion method, respectively. Despite there was no significant difference of total coliforms and *E. coli* population between residentials and home industries (P > 0.05) in this study, their density as well as prevalence remained high in the water sample. This might expose serious health risks since the resistance might be easily spread acquired through horizontal gene transfer within the aquatic environment.

1. Introduction

Antibiotic resistant bacteria (ARB), including *E. coli*, remain a major threatening problem, with concern increasing about their transmission throughout the environment. These bacteria persist causing infection even after the administration of current antibiotics. This leads to generating significant implications in both clinical and environmental settings.

Through horizontal gene transfer, ARB might disseminate their resistance traits to other bacteria, not only from the closed-related bacteria but also quite distantly bacteria. This dissemination prevalently occurs in environmental settings, especially in aquatic environments [1]. Aquatic environments are directly impacted by anthropogenic activities providing an ideal setting for the introduction and dissemination of ARB [1]. Not surprisingly, ARB is commonly present within the aquatic environment with highly dense population plus lack wastewater treatment facilities [2]. This is a consequence of higher loads of anthropogenic pollutants, including faecal contamination from human or animals associated with antibiotic resistance bacteria [3].

Banda Aceh is one of the growing cities in Indonesia with a population of almost 250,000 people in 2014 with nine major rivers flowing within the city. Similar to other growing cities, Banda Aceh faces typical urban problems with has no wastewater treatment plant currently installed with a variety
of human activities occur alongside the side, including residencies and home industries. Therefore, the objectives of the study were to enumerate, isolate, and determine (multiple) antibiotic resistance profiles and compare *E. coli* isolates recovered from residencies and home industries in Banda Aceh and its surrounding area.

2. Methods

2.1. *E. coli* enumeration and isolation

*E. coli* isolates were recovered from water samples collected from two sites of stream water, namely Jambo Aye river (5°32’19” N, 95°18’41” E) dominated by residencies and LuengPaga River (5°31’59” N, 95°19’30” E) dominated by home industries (tofu producers). On the designated sampling sites, approximately 300 mL of water sample was aseptically collected using sterile 500 mL Schott Duran bottles before they were transported on ice to the laboratory of Microbiology, Biology Department, Syiah Kuala University and examined within 24 h.

Examination of water samples was performed using Standard Total Coliform Multiple-Tube (MPN) Fermentation Method. Briefly, three series of 5 tubes each containing lactose fermentation broths were used as media for presumptive tests, whereas brilliant green lactose bile (BGLB) media for total coliforms and *E. coli* (EC) media were used for confirmatory tests. A 1-mL of water sample was transferred into each series of five tubes. The tubes were gently shaken to mix the sample with the medium before they were incubated for 48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C. After 18 or 24 hours, the positive tubes indicated by the increasing turbidity and gas production or a colour change were recorded. The tubes were then further incubated and re-examined after a total coliform of 48 hours. An inoculum of positive water was transferred onto tubes containing BGLB media for total coliforms and EC media for faecal coliforms. The test tubes were then incubated for 48 hours at 35 ± 0.5 °C or 37 ± 0.5 °C for total coliforms (BGLB broth) or 24 hours at 44 ± 0.5 °C for faecal coliforms (EC medium). After the designated incubation time, the positive tubes showing growth with the production of gas were recorded. A loopful of the positive gassing EC tubes in the presumptive test was inoculated on an eosin methylene blue (EMB) agar plate and incubated for 18-24 hours at 35 ± 0.5 °C. After incubation, *E. coli* colonies, i.e. dark centred flat with or without metallic sheen, were then transferred to plate count (PCA) agar slants before they were incubated for 18-24 hours at 35°C±0.5°C and subjected to biochemical identification, including the triple sugar iron (TSI) agar, Simmon-citrate agar, and Gram-staining procedures. A positive control of *E. coli* laboratory isolates was run in parallel with samples in the examination. A total coliform and *E. coli* were determined using most probable number (MPN) tables.

2.2. Antibiotic Resistance profile

The antibiotic susceptibility tests were performed using the disk diffusion method against four different antibiotics, namely ampicillin-clavulanate (30 µg); tetracycline (30 µg); gentamicin (10 µg); and nalidixic acid (30 µg). A single colony of *E. coli* isolates suspended in sterile five mL of NaCl 0.9% to make an equivalent McFarland 0.5 turbidity standard. The suspension was then evenly streaked on the surface of plates containing Mueller Hinton agar (MHA) using a sterile cotton swab. The disk(s) containing antibiotics with concentrations of breakpoint interpretive criteria established by the National Committee for Clinical Laboratory Standards (CLSI)[4] were then placed on the agar following incubation at 37 °C for 18-24 hr. The susceptibility tests were then determined, i.e., susceptible, intermediate, or resistant, using CLSI standards [4].

2.3. Data Analysis

A parametric test of two-sample t and z-test was conducted to evaluate the bacterial density of two sampling sites, namely residencies and home industry using XLSTAT 2016 (New York, USA). When appropriate, means will be separated by least significant difference (LSD) at α = 0.05. The results were also descriptively displayed in charts.
3. Results and discussion
The current study demonstrated that the bacterial density of log CFU, i.e., total coliforms and \textit{E}. \textit{coli} respectively, per 100 mL water sample collected from upstream, centre, and downstream of the river dominated by two different human activities, residential, and home industries (Figure 1a and 1b). The \textit{E}. \textit{coli} density of each sampling site between residential and home industries was not significantly different \((P = 0.339, \text{data not shown})\). However, the bacterial density per 100 mL of the water sample from both areas of residential and home industries was more than five orders of magnitude for both total coliforms and \textit{E}. \textit{coli}.

These findings in this investigation were considered higher than other similar studies collecting the water sample from the urban aquatic environment. Previous study collected total coliform and \textit{E}. \textit{coli} from stream water ranging from 0.27 – 4.17 log\textsubscript{10} CFU/ 100 mL and 0.17 – 2.54 log\textsubscript{10} CFU/ 100 mL, respectively [5]. Moreover, a separate study in the Semenyih River, Peninsular Malaysia, detected 649-219,250 CFU/ 100 mL of total coliform and 346.7-160,500 CFU/100 mL of \textit{E}. \textit{coli} [6]. The presence of total coliform and \textit{E}. \textit{coli} in high density in water samples of this study suggests that the stream water in this study was highly contaminated by total coliform and \textit{E}. \textit{coli} due to the faecal presence of human, warm-blood animals or other sources, such as wastewater discharges [7].

The occurrence of total coliforms and \textit{E}. \textit{coli} within the aquatic environment is more likely to prevalently emerge in accordance with increasing human activities. According to Indonesian authority for the water standards, total coliforms should not be detected more than 50 or 10 CFU per 100 mL for non-pipeline water or water-pipeline, respectively [8]. Moreover, World Health Organization require no total coliform and \textit{E}. \textit{coli} must be detected in any 100-mL sample [9]. Additionally, this study also indicates that there was faecal contamination in the stream water leading to an alarming health risk so that it justifies treating the water before it is utilized as source for daily activities, including drinking, bathing, and recreational purposes.

![Figure 1](image_url)

\textbf{Figure 1.} Bacterial density (log10 CFU/ 100 mL) of total coliform (A) and \textit{E}. \textit{coli} (B) from two locations, residential and home industries, at three sampling sites, upstream, centre, and downstream.

Of this study, there were a total of 43 \textit{E}. \textit{coli} isolates recovered from the water sample of both areas of residential and home industries. Among those isolates, there were 19 (44\%) \textit{E}. \textit{coli} isolates resistant to at least one of the tested antibiotics (ampicillin-clavulanate, tetracycline, gentamicin, and nalidixic acid). Figure 2 shows the proportion of the resistant \textit{E}. \textit{coli} isolates from stream water of residential and home industries in three sampling sites.
Figure 2. The proportion of antibiotic resistant *E. coli* (N = 43) from the water sample of two locations, residential (n = 15) and home industries (n = 4), at three sampling sites, upstream, center, and downstream. Numbers at the top of the chart indicate a subtotal of antibiotic resistant *E. coli* isolates from each sampling site.

In addition to be an indicator organism for faecal water contamination, *E. coli* has been also used for surveillance of antimicrobial drug resistance due to not only its prevalent occurrence in a wide range of host, including humans and animals [7], but also its easy resistance acquisition through horizontal gene transfer within aquatic environment [10]. Of this study, the proportions of *E. coli* isolates between those which were recovered from residential and home industries was not significantly different (*P* = 0.298, data not shown). However, this study suggests that there was a quite prevalence of antibiotic resistant *E. coli* in the aquatic environment. Other investigation found that total coliform and *E. coli* resistant to antibiotics were up to 96.4% [6], whereas others found up to 64% [5]. There are some factors affecting the prevalence and persistence antibiotic resistant total coliform and *E. coli* within aquatic environment [11]. This includes the increasing occurrence of organic and inorganic pollutants, including antibiotics particularly at the sub-inhibitory level [12], released into the environment and contributing to the proliferation of antibiotic resistant bacterial population through selective pressure. Another factor might involve heavy metals contamination facilitating co-selection for the resistant bacteria since, at molecular level, the genes responsible for the antibiotic resistance traits are commonly linked with heavy metal tolerance [13]. Moreover, the antibiotic resistance traits might be easily transfer trough conjugation if the corresponding genes are encoded within mobile genetic elements, such as plasmids [14] and integrons [15, 16]. In a different study, the occurrence of plasmids and integrons might contribute to the increasing number of multiple drug resistance among *E. coli* population [17]. Not surprisingly, antibiotic resistant genes and mobile genetic elements were then considered to be substantial environmental contaminants [18].

4. Conclusion
Despite there was no significant difference between total coliforms and *E. coli* population between residential and home industries in this study, their density as well as prevalence remained high in the water sample exposing serious health risks.

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