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Research

Water Quality and Prevalence of Extended Spectrum Beta Lactamase Producing *Escherichia coli* (ESBL *E. coli*) in Sungai Terengganu, Malaysia

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ABSTRACT

Antimicrobial resistance (AMR) is a significant global issue impacting human, animal, and environmental health, as well as economic security. Water bodies, especially, are now recognized for their role in the spread of antimicrobial-resistant pathogens, though standardized monitoring methods are lacking. This study investigates the presence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* in the Sungai Terengganu River in Malaysia, focusing on water quality and ESBL *E. coli* monitoring. Sampling was conducted twice in 2023 at 13 locations along the river. Overall, the water quality index (WQI) classified the river as Class II (WQI = 81), with upstream regions rated as Class I, indicating minimal anthropogenic impact. Higher Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), and Total Suspended Solids (TSS) in downstream areas suggested organic pollution from anthropogenic activities. Four metal elements exceeded the National Water Quality Standards (NWQS): Al (0.88 mg/L), Fe (3.45 mg/L), Cu (0.102 mg/L), and Hg (2.525 mg/L). No Pb, Cr, Se, Sn, or As were detected. Statistical analysis showed better water quality correlates with lower microbial contamination. ESBL *E. coli* was found in 8 of 13 sampling sites (61.5%), with the highest concentration at 800 CFU/100 mL. From 40 presumptive ESBL isolates tested, 87.5% (35 isolates) were positive for ESBL and carried additional drug resistance genes, including *mcr-1*, *tetW*, *sul-1*, and *sul-2*. These findings indicate a concerning presence of multidrug-resistant *E. coli* in the river, representing a potential public health risk.

Key words: Antimicrobial resistance (AMR), antimicrobial resistance genes (ARGs), environment, Malaysia, water

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INTRODUCTION

Antimicrobial resistance (AMR) has been acknowledged as one of the global threats. It is estimated that by 2050, more than 10 million people-could die annually due to infections caused by resistant microorganisms (O'Neill, 2016) AMR occurs when microbes become resistant to antimicrobial drugs, causing longer treatment processes and higher treatment costs. Besides posing life-threatening risks to humans and animals, the environmental sector has been identified as a key contributor to the emergence of AMR.

Water bodies, especially rivers, play a vital role as a source of freshwater for living organisms. Rivers serve not only as a source of drinking water supply, it are also important for economic activities such as irrigation, agriculture, aquaculture, and recreational activities. Anthropogenic activities mentioned above, however, may contribute to the deterioration of water quality status. The United Nations Environment Programme has outlined five main pollutant sources contributing to the transmission and spread of AMR in the environment; i) poor sanitation, sewage, and waste effluent; ii) pharmaceutical manufacturing waste; iii) healthcare facilities waste; iv) use of antimicrobial and manure in crop production; and v) animal production waste (United Nations Environment Programme, 2022). The climate change crisis worsens the scenario where contaminations from untreated sewage due to flooding and lack of clean water accessibility during drought contribute to the spread of AMR (Cavicchioli *et al*., 2019).

Studies on the prevalence of AMR in water bodies in Malaysia are still scarce compared to human and animal counterparts. This may be due to no specific regulations or monitoring for AMR from the effluent discharge and a lack of awareness of the impact on the environment. Bong *et al*. (2022) reported high concentrations of resistant *E. coli* were found in Sungai Larut, Perak where some of the isolates harbor multidrug-resistant genes (MDR). Antimicrobial-resistant bacteria (ARB) was also reported identified in other states in Peninsular Malaysia, Sabah, and Sarawak (Tissera & Lee, 2013; Kam *et al*., 2018; Salikan *et al*., 2020; Ho *et al*., 2021; Jamrin *et al*., 2022; Mohamad Hamdi *et al*., 2023). However, none of these reports focus on single indicator that may be used for AMR monitoring.

The World Health Organisation has been implementing a one-health AMR surveillance known as the Tricycle Project (World Health Organisation, 2021). In this project, Extended-Spectrum Beta-Lactamase-producing *E. coli* (ESBL *E. coli*) is used as an indicator to look at the relationship between humans, animals, and the environment towards AMR transmission. This pilot project was implemented in some countries such as Ghana, Pakistan, Malaysia, Madagascar, Senegal, and Indonesia (Puspandari *et al*., 2021, World Health Organisation, 2021). Therefore, in this study, we would like to monitor the presence of ESBL *E. coli* in water bodies in Malaysia. The water quality status of Sungai Terengganu, Terengganu was investigated for both chemical and microbiological parameters. The presumptive ESBL *E. coli* isolated from the selective media were characterized for ESBL phenotypic confirmation and detection of other ARGs. Positive ESBL *E. coli* isolates were then evaluated for harboring multiple resistance genes (ARGs).

MATERIALS AND METHODS

Sample collection and handling

Sungai Terengganu was chosen in this study because we would like to see the correlation between resistant bacteria with the water quality index. Sungai Terengganu has been categorized as Class II by the Department of Environment (Class II) since 2010. A total of 13 sampling locations were selected within the Sungai Terengganu for this study (Figure 1). The coordinates and land use activities are summarized in Table 1. Samples were collected twice in 2023 during the wet season (October & November 2023) using the grab sampling method. The samples were preserved according to the American Public Health Association (Bard *et al*., 2017). For microbiological analysis, samples were transferred to a 1L sterile glass bottles with screw caps. *In situ* parameters such as pH, temperature, stream flow, and visual appearance of water samples were recorded. Samples were then preserved in the cool box containing ice ensuring the temperature is below 10°C during transportation to the laboratory.

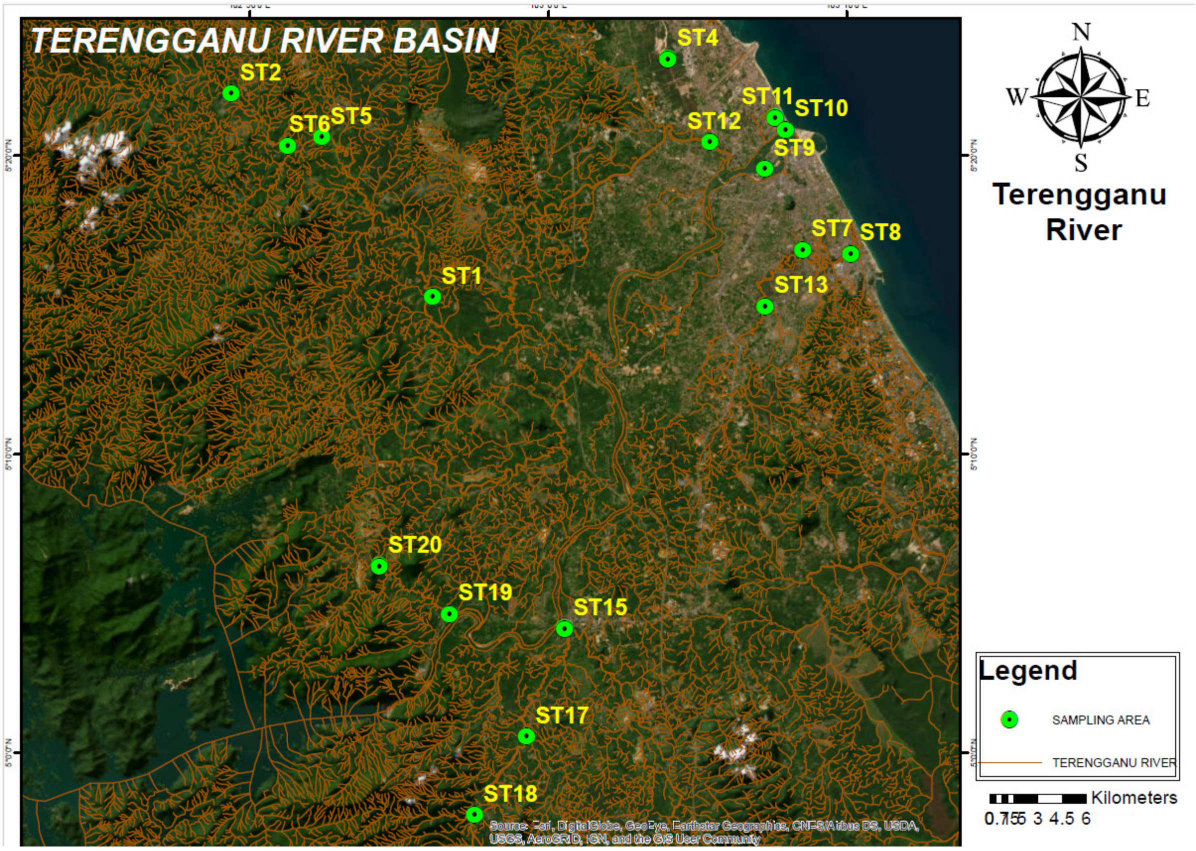
Water quality analysis

For water quality status, the samples were analyzed for Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammoniacal Nitrogen (AN), and Total Suspended Solid (TSS). Other parameters such as heavy metals, mercury, total nitrogen, and total phosphorous were also investigated. For in situ parameters (pH, dissolved oxygen (DO) & temperature) data were obtained during sampling using a YSI multi-parameter Water Quality probe.

Samples were processed accordingly using the reference method developed in the laboratory. For BOD, TSS, Heavy Metals, mercury, Fecal Enterococci, and Fecal coliform, analysis was carried out based on the American Public Health Association (APHA) 23rd edition (Bard *et al*., 2017). For COD, AN and total phosphorous, samples were processed based on the manufacturer protocol (HACH, USA). Analysis of total nitrogen was performed based on the manufacturer protocol (Shimadzu, Japan) whereas analysis of *Escherichia coli* and ESBL *E. coli* were performed based on WHO Tricycle protocol (World Health Organization, 2021). The water quality index (WQI) status of each location sites was calculated based on National Water Quality Standards (NWQS) Department of Environment guidelines as shown in Equation 1.

Equation 1:

$$WQI = [0.22 \times SIDO] + [0.19 \times SIBOD5] + [0.16 \times SICOD] + [0.15 \times SINH3 -N] + [0.16 \times SISS] + [0.12 \times SIpH]$$



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Fig. 1. Sampling location and coordination were conducted in this study.

Table 1. Coordinates and land use activities within the sampling site

Sampling Point	GPS Location	Location	Land use
ST20	5.103946, 102.905474	Bukit Kapah	Forest, Agriculture
ST19	5.077157, 102.944736	Kampung Kuala Kueh	Agriculture, Residential
ST18	4.965197, 102.958796	Sekayu	Forest, Recreational
ST17	5.008964, 102.987564	Kampung Kuala Menjing	Forest, Agriculture
ST15	5.06905, 103.008813	Kuala Berang	Residential
ST12	5.340640, 103.090040	Kampung Gelanggi	Residential
ST11	5.354419, 103.126278	Ulu Takir	Recreational
ST10	5.347538, 103.132166	Seberang Takir	Recreational
ST9	5.325700, 103.120538	Pualu Duyong	Commercial
ST8	5.278361, 103.168626	Sungai Ibai	Commercial, Residential
ST13	5.248893, 103.12082	Kampung Se Rendang	Residential
ST7	5.280528, 103.141727	Kampung Laut	Residential
ST4	5.386993, 103.066496	Kuala Nerus	Commercial, Residential

Enumeration of presumptive ESBL *E. coli*

Analysis was carried out based on the WHO Tricycle Protocol (World Health Organization, 2021). Briefly, three different water sample volumes ranging from 10 – 100 mL were filtered through a 0.45 μm membrane filter and incubated using Tryptone Bile X-glucuronide (TBX) agar supplemented with 4 μg/mL of cefotaxime. Samples were also incubated using media without any antimicrobials to calculate the prevalence of ESBL *E. coli/E. coli* in the samples. The prevalence of ESBL *E. coli/E. coli* was calculated based on equation 2 below.

Equation 2:
Prevalence (%) = (total count ESBL *E. coli* (CFU/100 mL)/(total count *E. coli* (CFU/100 mL) × 100

ESBL phenotypic confirmation

A total of 5 ESBL *E. coli* colonies were randomly picked from each sampling site. Each isolate was subcultured onto TBX Agar supplemented with 4 µg/mL cefotaxime until the pure culture was obtained. An indole test was performed for each pure isolate for strain confirmation.

For ESBL *E. coli* phenotypic confirmation, a Combination Disk Diffusion Test (CDT) was performed based on CLSI and EUCAST guidelines (Giske *et al*., 2017; CLSI, 2020). Four discs of antibiotic discs; cefotaxime (CTX), ceftazidime (CAZ), cefotaxime/clavulanic acid (CTX/CLA) and ceftazidime/clavulanic acid (CAZ/CLA) were placed on a Mueller Hinton agar lawned with 0.5 MacFarland isolated suspension and further incubated at 35°C for 16 – 18 hr. The strain is considered as positive ESBL if the zone diameter of the combination disc (CTX – CTX/CLA or CAZ – CAZ/CLA) is more or equal to 5 mm. A non-ESBL *E. coli* strain (ATCC 25922) and ESBL *E. coli* strain (CDC AR-0346) were used in this method as a quality control.

Detection of antimicrobial resistance genes (ARGs)

To further characterize the genetic elements involved in the resistance mechanisms, PCR analysis was conducted to screen for ARGs from the positive ESBL isolates. Sixty positive isolates were screened for the presence of *TEM*, *SHV*, *sul-1*, *sul-2*, *mcr-1,* and *tetW* genes. Genomic extraction for the isolates was carried out using the Primeway DNA Extraction Kit (Apical Scientific Sdn. Bhd.) based on the manufacturer's protocol. Two control strains were used in this analysis; *E. coli* ATCC 25922 (negative control) and *E. coli* CDC AR-0346 (positive control). The list of primers used in the analysis is summarized in Table 2. The PCR was performed in a 50 µL containing 1X PCR Master Mix (QIAGEN, Germany), 0.2 µM primers, and 10 ng of template. PCRs were performed as follows: Initial denaturation was at 94°C for 3 min followed by 29 cycles of denaturation at 94°C for 45 sec, annealing at 58 - 60°C for 30 sec, and elongation at 72°C for 1 min with final extension at 72°C for 15 min.

Table 2. Primer sequence and its annealing temperature for ARGs detection

Target genes	Sequence (5' – 3')	Annealing temperature (°C)	Reference
TEM	F- TCCTTGAGAGTTTTCGCCCC R- TGACTCCCCGTCGTGTAGAT	60	Kamaruzzaman *et al*. (2020)
SHV	F – CAATCACGACGGCGGAATCT R - GTGGGTCATGTCGGTACCAT	60	Kamaruzzaman *et al*. (2020)
sul-1	F -CCGTTGGCCTTCCTGTAAAG R - TTGCCGATCGCGTGAAGT	60	Heuer & Smalla (2007)
sul-2	F - CGGCTGCGCTTCGATT R - CGCGCGCAGAAAGGATT	60	Heuer *et al*. (2008)
tetW	F - CGGCAGCGCAAAGAGAAC R - TTTGGCGTGTTTCATTGCTT	60	Walsh *et al*. (2011)
mcr-1	F - AGTCCGTTGTTCTTGTGGC R - AGATCCTTGGTCTCGGCTTG	58	European Centre for Disease Prevention and Control (2019)

RESULTS

Water quality analysis

Physiochemical parameters

Overall, the Water Quality Analysis (WQI) value for Sungai Terengganu remained at Class II (WQI = 86) indicating that it is not polluted. Based on the National Water Quality Standard (NWQS), Class II water requires conventional treatment before being used and is safe for recreational activities with body contact. All sampling locations were classified as Class II with WQI values ranging from 78 to 92. Only ST18, which is located in the upstream region of Sungai Terengganu recorded Class I water quality with a WQI value of 95. However, sampling location ST8 in the downstream region was classified as Class III (slightly polluted) with the lowest WQI value of 54.

Analysis of physiochemical parameters at all sampling sites recorded values within the limit range for Class III except for certain parameters such as Total Suspended Solids (TSS) and Ammoniacal Nitrogen (AN). The Biochemical Oxygen Demand (BOD) values recorded in Class II (threshold = 6 mg/L) were compared to NWQS standards. The highest BOD value was recorded at ST8 (4.2 mg/L), while the lowest BOD values were recorded at ST1 and ST15 (1 mg/L) (Figure 2(a)). For Chemical Oxygen Demand (COD), all sampling sites recorded values within Class II (threshold less than 25 mg/L), with the highest value recorded at ST8 (22.85 mg/L) and the lowest at ST18 (2.5 mg/L) (Figure 2 (b)). The same trend was observed for TSS, with all location sites categorized as Class II (not exceeding 50 mg/L) except of ST4 (169 mg/L), ST8 (155 mg/L) and ST15 (153 mg/L), where the values recorded fell into Class IV (threshold = 300 mg/L) (Figure 2 (c)). The pH values recorded were within the acceptable range for Class II (6.0 – 9.0) except for ST4 (pH = 4.61). The highest pH was recorded from the ST10 sample (pH =7.05) (Figure 2 (d)). The Ammonia Nitrogen (AN) values were within the Class III range (not exceeding 0.9 mg/L) except for ST15 (3.42 mg/L), ST8 (2.35 mg/L), and ST4 (6.45 mg/L), where the values were considered as polluted in Class V (Figure 2 e)). As for Dissolved Oxygen (DO), the average values recorded were within Class II (range 5 – 7 mg/L), with the highest DO value recorded at ST18 (8.8 mg/L) and the lowest DO value at ST17 (6.15 mg/L) (Figure 2 (f)).

Apart from the water quality index parameter, other chemical parameters such as total nitrogen, total phosphorous, and heavy metals were also evaluated. The highest concentration of phosphorous was recorded at ST17 (1.23 mg/L), and the lowest was observed at ST9 (0.23 mg/L) (Figure 2 (g)). For total nitrogen, the highest concentration was recorded at ST20 (2.74 mg/L), while the lowest was observed at ST18 (0.28 mg/L) (Figure 2 (h)).

Nineteen heavy metal elements were screened in this study; Lead (Pb), Zinc (Zn), Aluminium (Al), Nickel (Ni), Chromium (Cr), Iron (Fe), Boron (B), Copper (Cu), Selenium (Se), Silver (Ag), Tin (Sn), Barium (Ba), Manganese (Mn), Cadmium (Cd), Arsenic (As), Magnesium (Mg), Calcium (Ca), Natrium (Na) and Mercury (Hg). Among these, only four elements exceeded the NWQS standards: Al, Fe, Cu, and Hg. The highest concentration of Al was detected at ST17 (0.88 mg/L); Cu at ST18 (0.102 mg/L); Fe at ST15 (3.45 mg/L); and Hg at ST19 (2.525 mg/L). No Pb, Cr, Se, Sn, and As elements were detected in all sampling sites.

Microbiological parameters

Apart from *E. coli* and ESBL *E. coli* analysis, fecal coliform and fecal enterococci analysis were also performed (Figure 3). Fecal coliform was detected in a range of 300 – 27,000 CFU/100 mL, with ST10 site recorded the lowest concentration and ST7 recorded the highest concentration. Two sampling sites exceeded the threshold limit from NWQS (20,000 CFU/100 mL) which were ST7 (27,000 CFU/100 mL); and ST8 (21,000 CFU/100 mL). For fecal enterococci, ST4 recorded the lowest concentration (200 CFU/100 mL) and ST15 recorded the highest concentration (9,000 CFU/100 mL).

For *E. coli*, the lowest concentration was detected at ST4 (5 CFU/100 mL), and the highest concentration was detected at ST8 (7,700 CFU/100 mL). No ESBL *E. coli* was detected at five sampling sites which are ST20, ST17, ST18, ST19, and ST4. The highest concentration of ESBL *E. coli* was detected at ST11 (800 CFU/100 mL). The prevalence of ESBL *E. coli/E. coli* for Sungai Terengganu for this study was 61.5 %.

ESBL phenotypic confirmation and ARGs screening

A total of 40 ESBL *E. coli* isolates (5 isolates for each location) were tested for phenotypic confirmation using the Combination Disc Diffusion Test (CDT). From the analysis, only 35 (87.5%) of the isolates were positive for ESBL (Figure 4). The positive ESBL isolates were then used for multi-drug resistance gene screening. Table 3 below shows the summary of ARGs screened using PCR. Overall, almost 95% of the isolates tested carry tetracycline resistance gene (*tet*W), followed by sulfonamide resistance genes (*sul-2*) (92%) and other beta-lactamase resistant genes; bla_{CTX-M} (88%), bla_{TEM} (88%) and bla_{SHV} genes (82%). However, *mcr-1* resistant genes; and carbapenemase resistant genes were not detected in any of the isolates tested.

DISCUSSION

The water quality index (WQI) is a numerical method used to classify rivers based on six main parameters; Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Ammonia Nitrogen (AN), Total Suspended Solids (TSS), pH and Dissolved Oxygen (DO). A higher WQI value indicates a highly preserved river quality. Sungai Terengganu, Malaysia has been considered clean (Class II) since 2010 based on water quality monitoring by the Department of Environment (2010). However, this river serves several economic activities such as tourism, fisheries, agricultural plantations, commercial industries, urban and rural residential areas, and forestry that may indirectly impact water quality (Suratman *et al*., 2015). In this study, sampling was conducted during the wet season (October – November 2023), with average rainfall during this season exceeding 200 mm.

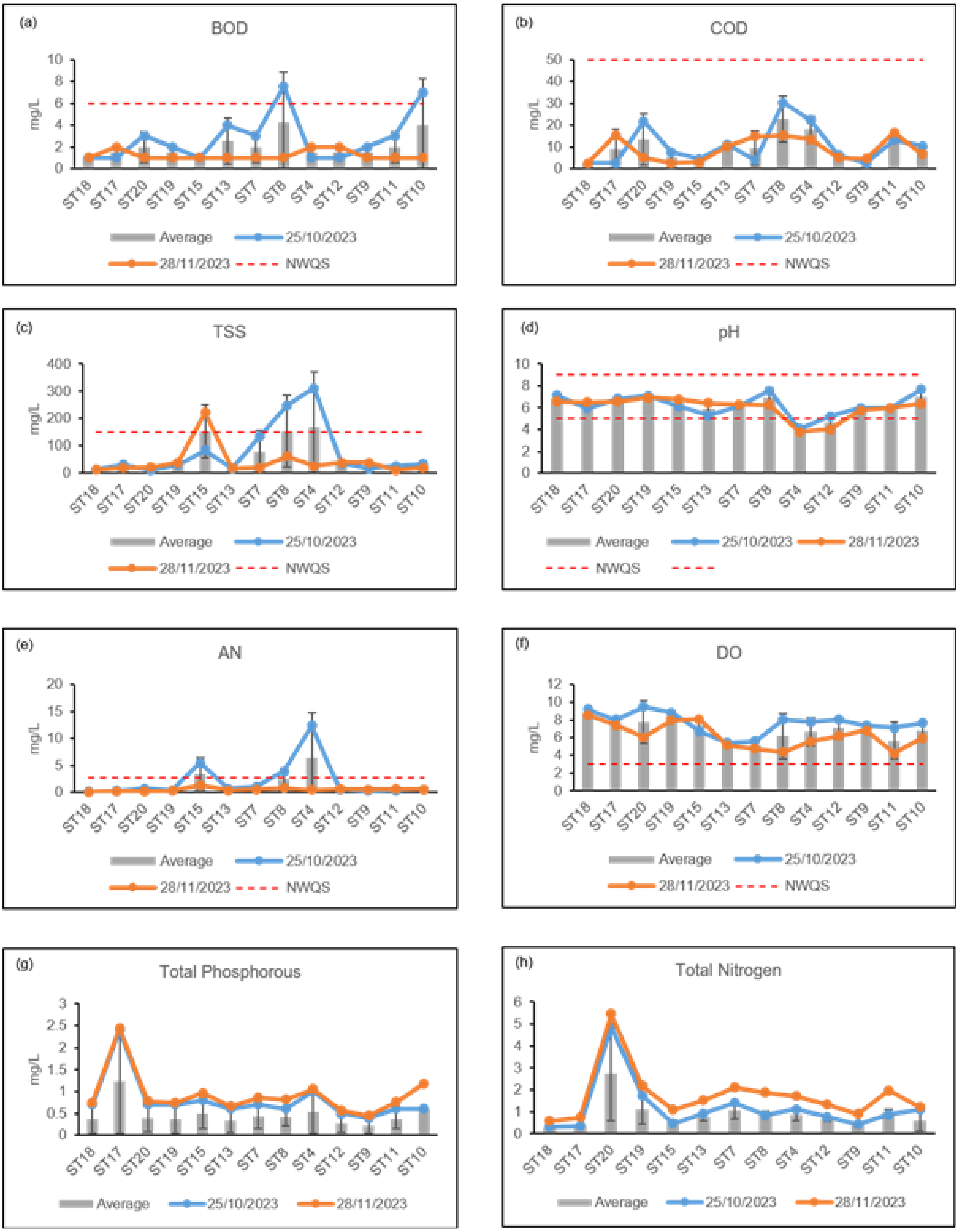


Fig. 2. Water quality parameter analysis for 13 sampling sites in Sungai Terengganu. (a) BOD analysis; (b) COD analysis; (c) TSS Analysis; (d) pH analysis; (e) AN analysis; (f) DO analysis; (g) Total phosphorous analysis; (h) Total nitrogen analysis.

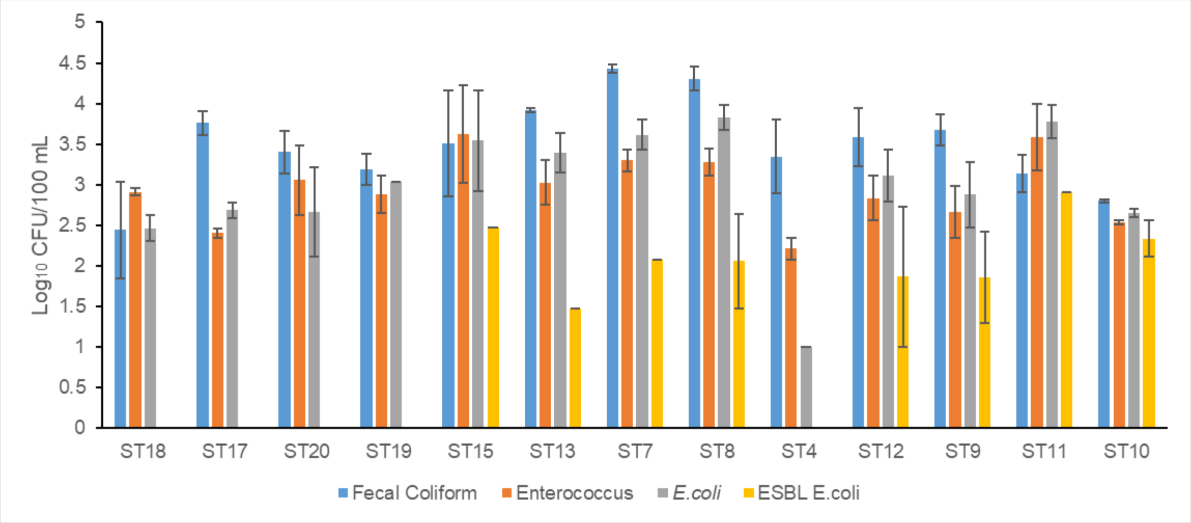


Fig. 3. Microbiological analysis for 13 sampling sites in Sungai Terengganu.

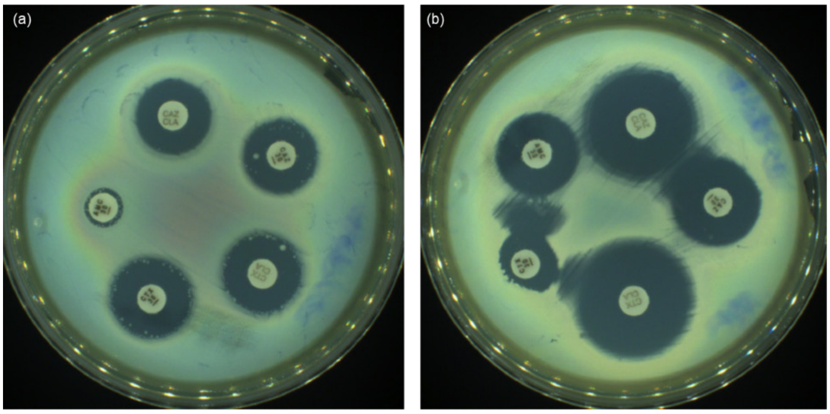


Fig. 4. Combination Disk Diffusion Test (CDT) for ESBL *E. coli* phenotypic confirmation. (a) Antibiogram for negative ESBL and (b) for positive ESBL.

Table 3. Prevalence of different ARGs detected from ESBL *E. coli* isolates

Prevalence (%)	*bla*$_{CTX-M}$	*bla*$_{SHV}$	*bla*$_{TEM}$	*mcr-1*	*sul-1*	*sul-2*	*tetW*
ST15 (*n*=2)	50	100	50	0	50	50	100
ST13 (*n*=5)	100	100	80	0	80	80	80
ST7 (*n*=5)	80	100	100	0	80	80	100
ST8 (*n*=4)	100	100	50	0	100	100	75
ST12 (*n*=5)	100	100	80	0	80	80	100
ST9 (*n*=4)	100	100	100	0	100	100	100
ST11 (*n*=5)	80	60	80	0	80	80	100
ST12 (*n*=5)	80	60	100	0	100	100	100
Total (*n*=35)	**88.57**	**88.57**	**82.86**	**0**	**85.71**	**91.43**	**94.29**

Our sampling locations were divided into two main areas; upstream (ST15, ST17, ST18, ST19, ST20) flowing from Kenyir Lake to downstream locations near Kuala Terengganu (ST4, ST7, ST8, ST9, ST10, ST11, ST12, ST13), which flows into the South China Sea. Minimal anthropogenic activities in the upstream area may contribute to the high WQI value and other favorable water quality parameters compared to the downstream area. Key parameters for WQI in the upstream area are categorized under Class I; indicating-water that is conserved within its natural environment. For instance, sampling site ST18, located in Sungai Sekayu recreational area is highly visited for recreational activities due to its clear and pristine water. Therefore, it is crucial to preserve this area from contamination sources that could lead to water-borne related diseases, including AMR. On the other hand, water quality deteriorates in the downstream region, especially at ST8 and ST4, evident in parameters such as BOD, COD, and TSS. Land use activities (constructions) observed in the ST4 area may contribute to high TSS levels due to soil erosion entering the river. Because the area is densely populated with residential areas, elevated BOD and COD values at ST8 suggest that organic pollution may be coming from residential areas.

The NWQS WQI calculation does not incorporate microbiological parameters, which means that the calculated water quality index may not fully represent the actual quality of the river tested (Uddin *et al*., 2021). Based on Pearsons' correlation analysis in this study, there are negative correlations between WQI values and microbiological indicators (fecal coliform, fecal enterococci and *E. coli*); higher water quality represents to lower concentrations of microbial contaminations as per recorded in the upstream area (ST118 & ST20) where the influence of anthropogenic activities are low. Chemical parameters like BOD, COD, TSS and pH shows positive correlations with microbiological parameters. Low BOD values indicate lower microbial counts in the samples, as they consume oxygen to degrade organic matter, resulting in higher dissolved oxygen levels. Higher Total Phosphorus (TP) and Total Nitrogen (TN) values are contributed mainly from agriculture waste, disrupting the aquatic ecosystem leading to harmful algal blooms and deterioration in water quality (Shen *et al*., 2021). This can be seen in ST17 and ST20 where high concentration of TP and TN detected in this area that might be due to oil palm plantation that covers the majority of the land use in this area. A significant decrease in the bacterial community has been observed in the Yangtze River Delta of China, particularly in Shanghai and Hangzhou Bay due to the high nitrogen and phosphorous loads in the river. These elevated nutrient levels can disrupt the ecological balance in the environment (Su *et al*., 2018; Lin *et al*., 2019). This disruption may lead to changes in microbial populations and potentially impact the overall health and functioning of the aquatic ecosystem in these regions.

The rationale for choosing ESBL *E. coli* as the key indicator for AMR transmission is due to the fact this strain has been isolated in other contexts from human health and animal health (Lim *et al*., 2009; Ho *et al*., 2012; Kamaruzzaman *et al*., 2020; Lemlem *et al*., 2023). The *E. coli* resides in the normal gut microbiota in humans and it is a major cause of both hospital-acquired and community-onset bacterial infections (Hernandez-Pastor *et al*., 2023). In animal settings, *E. coli* serves as a critical hygiene indicator for verifying processes in food production, alongside coliforms (Hariri, 2022) Moreover, it can be found in water and soil environments and is widely used as an organism to assess the quality of water in a specifc area (Bürgmann *et al*., 2018). Given its widespread presence and clinical significance, ESBL *E. coli* serves as a valuable marker for tracking antimicrobial resistance (AMR) transmission across various ecosystems and sectors.

ESBL *E. coli* was detected in eight of the sampling sites at a relatively low concentration (with a maximum concentration of 800 CFU/ 100 mL). The upstream areas remain unpolluted with the resistant bacteria since there is no absence of anthropogenic activities. An exception for ST4, where no ESBL *E. coli* was detected in this sampling area although it is located in one of the big cities in Terengganu. Through observation during sampling, this area may not be affected by any pollutants contributing to AMR since the area is in development for commercial use. To date, there are still no guidelines for the permissible concentration of ESBL *E. coli* in the environment sector as it is not being monitored routinely in the national environmental water quality monitoring program.

ESBLs are enzymes that can hydrolyze the expanded spectrum β-lactamases antibiotics such as ceftazidime and cefotaxime and inhibition by clavulanate (Castanheira *et al*., 2021). According to Jacoby (2006) , there are 12 families of ESBL enzymes which differentiate based on the substrates, biochemical properties, peculiarities of sequence, location of their discovery, location of the gene on the chromosome, strains of bacteria, the patient providing a sample, or the investigators who described them. ESBLs families *TEM*, *SHV,* and *CTX* are the most commonly isolated from the Gram-negative bacteria. This can be seen in our findings that more than 80% of the isolates harbour *TEM*, *SHV* and *CTX* genes. The high percentage of detection for *tetW* and *sul* genes may related to the antibiotic prescription practices as these two antibiotics are broad spectrum antibiotics that is commonly prescribed to treat infection. The presence of multiple resistance genes in all the isolates tested indicating the isolates may be resistant to multiple antibiotics. It has been acknowledged environment is not only one of the key factors for AMR transmission; but also as a source of acquisition of ARGs by pathogens. ARGs can be shared among the pathogens via horizontal gene transfers creating a vast genetic modification to the pathogen itself (Larsson & Flach, 2022). Scientific evidence has proved antimicrobial residue pollutions enhance a selection pressure which can contribute to resistance evolution. In this study, we found sulphonamides and tetracycline-resistant genes were detected in almost all the isolates tested.

This may be due to these antibiotics being commonly used in livestock and healthcare facilities. The same trend was observed in Beijiang River, South China where elevated levels of sulphonamides and tetracycline resistance genes were detected in highly urbanized areas (Ling *et al*., 2013). The evolution of colistin resistance genes has been one of the global concern as colistin antibiotic is one of the few available drugs for treating infections caused by carbapenem-resistant Enterobacteriaceae (Wang *et al*., 2018). From the PCR analysis, we did not detect any colistin-resistant isolates from this study. However, the analysis was only based on *mcr-1* genes; which may not be strong evidence to support the ESBL isolates do not co-exist with colistin-resistant genes.

CONCLUSION

The water quality of Sungai Terengganu was determined to be Class II, indicating that conventional water treatment is required for the water supply. Physicochemical and microbiological analysis showed that all parameters did not exceed the threshold limit for Class III, except Total Suspended Solids (TSS) parameters at the downstream region (ST4). Correlation analysis showed that high water quality represents the low concentration of microbial contaminations. The prevalence of ESBL *E. coli* in Sungai Terengganu calculated to be 61.5%, with no ESBL *E. coli* detected in the upstream region. Analysis of Antimicrobial Resistance Genes (ARGs) showed that all presumptive ESBL isolates carried more than one resistant gene. Further analysis on whole genome analysis and residual antibiotics may help in a better understanding of AMR transmission in the environment. The results obtained from this study may be used as a baseline data for the stakeholders to take action towards preserving the water resources in order to guarantee a continuous supply of clean and safe water. The spread of resistant bacteria in the environment may be halted by taking simple steps like refraining from disposing of trash that contains antibiotics in waterways.

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ETHICAL STATEMENT

Not applicable

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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