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Correlation of urban and rural settlements with *Shigella sp*. resistance pattern isolated from Bedadung River in Jember, Indonesia

Dimas Widyadhana Bhanu Aryasatya[1], Dini Agustina[2]*, Muhammad Afiful Jauhani[3], Enny Suswati[2]

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[1]Faculty of Medicine, Jember University, Jember, Indonesia

[2]Department of Microbiology, Faculty of Medicine, Jember University, Jember, Indonesia

[3]Department of Forensic and Medicolegal, Dr. Soebandi Regional Hospital, Jember, Indonesia

***Correspondence:**
dini_agustina@unej.ac.id

Abstract

Purpose: This study aims to determine a correlation between settlement location and resistance patterns of *Shigella* sp. isolated from the Bedadung River in Jember, Indonesia. **Method:** The research method used was analytic observational with a cross-sectional design. Data analysis was performed using the Chi-Square test and, if not qualified, the Fisher exact test. Samples were obtained through a total sampling technique with a total sample size of 24. **Results:** Most samples were resistant to the antibiotic, and there was no relationship between residential location and resistance patterns of Shigella sp. isolated from Bedadung River, Jember Regency (p-value > 0.05). **Conclusions:** In conclusion, no significant relationship exists between the residential area and the resistance pattern of *Shigella* sp. isolated from Bedadung River, Jember Regency. *Shigella* sp. bacterial contamination is mainly found in Bedadung River water isolates in rural and urban areas. *Shigella* sp. bacteria found in rural and urban areas in the Bedadung River in Jember Regency have the same number.

Keywords: antibiotic resistance; rural settlements; *Shigella* sp.; urban settlements

INTRODUCTION

One advancement in medicine is using antibiotics to treat infectious diseases. Antibiotic resistance, however, is already causing some issues and is predicted to result in 10 million yearly deaths by 2050 [1]. Horizontal gene transfer, or HGT, can more easily transmit antibiotic resistance in various media, including water, soil, air, food, and living organisms [2,3]. In contaminated aquatic habitats, such as rivers, antibiotic-resistant bacteria can spread quickly and become a source of illness. According to Ekwanzala et al. (2017), *Shigella* sp. is one of the most antibiotic-resistant bacteria that causes water-borne disease epidemics worldwide [4]. Resistance of *Shigella* sp. to numerous medications, including cotrimoxazole and ciprofloxacin, has increased by 60% globally, such as cotrimoxazole, ciprofloxacin, and ceftriaxone [5].

Previous studies have established that rivers in developing regions are hotspots for bacterial contamination, particularly in areas where urban and rural waste disposal systems are not adequately regulated. Shigella spp. contamination of water bodies has been well-documented, especially in countries with poor water treatment facilities. However, research has predominantly focused on bacterial loads and antibiotic resistance in urban rivers or regions near high-density populations. Few studies have examined the differential impacts of urban versus rural settlements on the antibiotic resistance profiles of Shigella spp. in river systems, particularly in regions like Jember, where diverse land use patterns coexist. Urban areas typically contribute significantly to river pollution due to higher population density, domestic sewage, and industrial effluents. Numerous studies indicate that urban rivers harbor higher concentrations of antibiotic-resistant bacteria due to extensive antibiotic use in healthcare, residential, and agricultural sectors [6,7]. Rural areas, particularly in agrarian zones, are not accessible due to the problem of river contamination. Agricultural runoff, which includes animal waste and improperly managed waste disposal systems, also introduces pathogenic bacteria and antibiotic residues into water bodies. Rural settlements may contribute to antibiotic resistance in rivers by using antibiotics in livestock farming, which promotes the spread of resistant bacterial strains [8].

Rapid urban and rural development may lead to slum areas with poor sanitation facilities. The threat of environmental antibiotic-resistant bacteria and waterborne disease transmission can rise as a result. Due to the dumping of different wastes, including household, industrial, agricultural, animal, and fisheries wastes, rivers have the potential to be contaminated and turn into a vehicle for disease transmission [2]. In various rivers in China's rural and urban regions, anthropogenic community activities have been linked to the spread of antibiotic-resistant bacteria in prior research [9]. While the relationship between urbanization, bacterial contamination, and antibiotic resistance has been well explored, the comparative analysis of urban and rural settlements' influence on bacterial resistance profiles in the same river system remains underexplored.

Furthermore, research on the correlation between settlement types and Shigella sp. resistance patterns is scarce. This gap is particularly critical in areas like Jember, Indonesia, where the river passes through urban and rural landscapes. Understanding how these different land use types affect bacterial resistance in river systems could offer new insights into environmental health and public safety [10].

The Bedadung River is a vital waterway in the Jember region of East Java, Indonesia, flowing through diverse settlements, including urban and rural areas. This river plays an essential role in the daily lives of local communities, serving as a water source for domestic, agricultural, and industrial use. However, its proximity to densely populated urban districts and rural agrarian areas makes it vulnerable to various contaminants, including pathogens such as *Shigella* sp. and antibiotic residues. The selection of this river is thus crucial for understanding how human activities associated with different types of settlements impact the water quality and, more specifically, the antibiotic resistance of pathogens within the ecosystem [11]. The comparison between urban and rural areas is vital due to distinct contamination sources: urban areas are more affected by industrial and domestic waste.

In contrast, rural regions contribute through agricultural runoff and animal waste. This distinction helps target solutions for river pollution management. Different environmental factors in urban and rural settings may also influence antibiotic resistance in *Shigella* sp. Studying this variation is crucial for understanding public health risks, as rivers passing through both areas can spread resistant bacteria. The Bedadung River offers a natural setting to explore how each settlement type affects bacterial resistance, informing strategies for public health and environmental management [12]. So *Shigella* sp. isolated from the Bedadung River, Jember Regency, was studied to ascertain the association between site and resistance patterns.

METHODS

An analytical observational study with a cross-sectional design was conducted at the Microbiology Laboratory Faculty of Medicine, University of Jember, from September to November 2022. Data from the results of the number of *Shigella* sp. bacteria isolated from rural and urban settlement areas fed by the Bedadung River water and their resistance test results using the Kirby Bauer disc diffusion method were collected. The inclusion criteria were data from 16 sub-districts drained by the Edadung River (Panti, Sukorambi, Jelbuk, Arjasa, Patrang, Sukowono, Sumberjambe, Ledokombo, Pakusari, Sumbersari, Kaliwates, Ajung, Rambipuji, Balung, Wuluhan, and Puger). Exclusion criteria in this study were *Shigella* sp. isolates of Bedadung River water that did not grow on Salmonella Chromogenic Agar media. Data were chosen using a total sampling, with a total sample size of 24 data (12 samples from rural areas and 12 samples from urban areas).

The tools used in this study were an inoculation loop, micropipette, digital scale, autoclave, binocular microscope, caliper, 35-37°C incubator, oven sterilizer, object glass, vortex, laminar airflow, and electric stove. Salmonella Chromogenic Agar media, Nutrient Agar media, Muller Hinton Agar media, sterile distilled water, water for irrigation, Gram staining instruments, antibiotic discs (azithromycin, erythromycin, sulfamethoxazole, gentamycin, amikacin, levofloxacin, cefotaxime, ceftriaxone, cefepime, penicillin, and amoxiclav) was used for materials in this study.

The diameter of the inhibition zone was assessed using a vernier caliper. Research data were tabulated into a Google Spreadsheet worksheet and statistically analyzed using the Statistical Package for Social Sciences for Windows. This study adhered to Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines for reporting inhibition zone diameter of resistance tests to antibiotics categories [13,14]. Inhibition zones were divided into susceptible, intermediate, and resistant.

The Chi-Square and Fisher's exact test were used to analyze the correlation between settlement location and resistance patterns of *Shigella* sp. isolated from the Bedadung River. This study was approved by the Ethics Committee of the Faculty of Medicine, University of Jember, with letter number 1686/H25.1.11/KE/2023.

RESULTS

This study involved 12 samples from rural and 12 from urban areas, as shown in Figure 1. The result showed that 24 (40%) SCA media of the 60 water isolates from the Bedadung River were positive for *Shigella* sp.

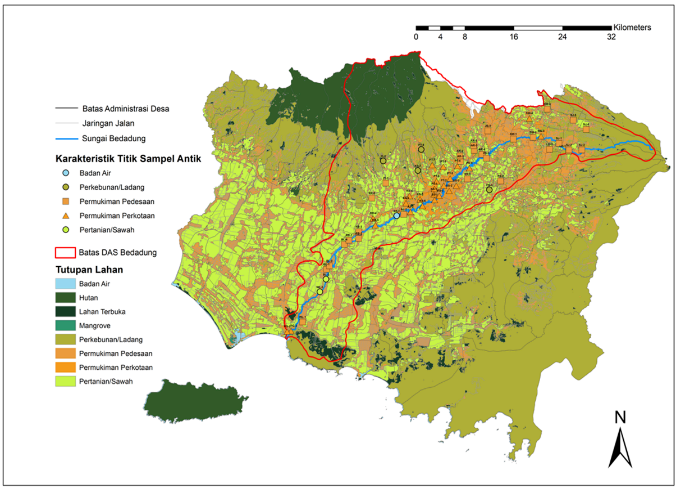

Figure 1. Map of Bedadung River water isolate collection location (source: Dinas Pekerjaan Umum Bina Marga dan Sumber Daya Air Kabupaten Jember)

After being identified, Shigella sp. was confirmed using Gram stain. Based on the Gram stain test results, 24 (100%) samples of *Shigella* sp. were from colonies of gram-negative coccobacillus bacteria or round rods, as seen in Figure 2. Antibiotic resistance test results on 24 samples of *Shigella* sp., 4 (16.67%) samples were resistant to azithromycin, 8 (33.3%) erythromycin, 2 (8.33%) amikacin, 1 (4.16%) gentamicin, 7 (29.16%) sulfamethoxazole, 3 (12.5%) levofloxacin, 9 (37.5%) amoxiclav, 11 (45.83%) ceftriaxone, 12 (50%) cefepime, 20 (83.3%) penicillin, and 17 (70.83%) cefotaxime. Figure 3 provides an example of the antibiotic resistance test results.

However, as shown in Table 1, there was no significant correlation between the location of settlements and the *Shigella* sp. resistance pattern isolated from the Bedadung River in Jember, Indonesia (p-value > 0.05).

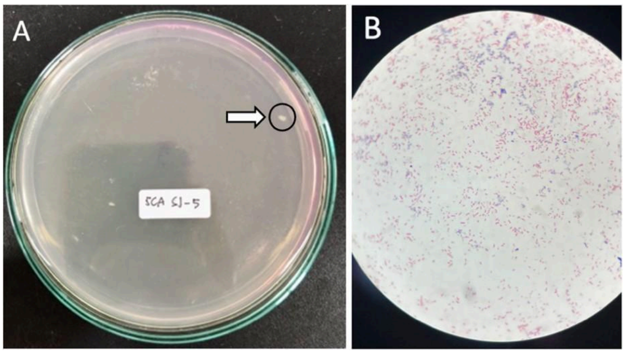

Figure 2. (A) Isolation of *Shigella sp*. on SCA media and (B) identification of Gram stain at 1,000x magnification

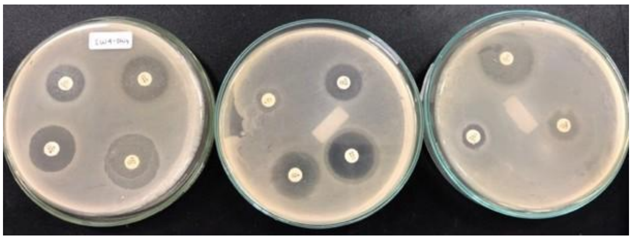

Figure 3. Resistance test result of *Shigella sp*. on Mueller-Hinton agar media

Table 1. Statistical analysis correlation between urban and rural settlement with Shigella sp. resistance pattern isolated from the Bedadung River in Jember, Indonesia

Antibiotic	Resistance pattern						Total	Sig.
	Susceptible		Intermediate		Resistant			
	n	%	n	%	n	%		
Azithromycin								
Urban	9	75	0	0	3	25	12	0.590
Rural	11	91.67	0	0	1	8.33	12	
Erythromycin								
Urban	4	33.3	3	25	5	41.7	12	0.251
Rural	2	16.7	7	58.3	3	25	12	
Amikacin								
Urban	10	83.3	0	0	2	16.7	12	0.478
Rural	12	100	0	0	0	0	12	
Gentamicin								
Urban	10	83.3	1	8.33	1	8.33	12	0.513
Rural	10	83.3	2	16.7	0	0	12	
Sulfamethoxazole								
Urban	8	66.7	1	8.33	3	25	12	0.565
Rural	8	66.7	0	0	4	33.3	12	
Levofloxacin								
Urban	12	100	0	0	0	0	12	0.217
Rural	9	75	0	0	3	25	12	
Amoxiclav								
Urban	3	25	5	41.7	4	33.3	12	0.686
Rural	4	33.3	3	25	5	41.7	12	
Ceftriaxone								
Urban	3	25	3	25	6	50	12	0.673
Rural	5	41.7	2	16.7	5	41.7	12	
Cefepime								
Urban	6	50	2	16.7	4	33.3	12	0.189
Rural	2	16.7	2	16.7	8	66.6	12	
Penicillin								
Urban	1	8.33	2	16.7	9	75	12	0.333
Rural	1	8.33	0	0	11	91.67	12	
Cefotaxime								
Urban	5	42.7	0	0	7	58.3	12	0.123
Rural	1	8.33	1	8.33	10	83.3	12	

DISCUSSION

This study showed that urban and rural settlements were not statistically significant with *Shigella* sp. resistance patterns isolated from the Bedadung River in Jember, Indonesia. Interestingly, a study conducted by Zhang et al. (2022) in China concerning the relationship between socioeconomic factors in rural and urban areas and quinolone antibiotic resistance showed insignificant results ($p > 0.05$). Rapid urbanization and increased human activity over the past few decades have caused ARGs to appear, develop, and spread across the surrounding environment in China. Urbanized regions and pristine locations, for instance, have significantly different ARG compositions in the Yarlung Tsangpo River, China, showing that human activities were crucial to the variety of ARGs [9]. In Indonesia, the geographical conditions of rivers, such as the Bedadung River, play a significant role in Shigella sp's contamination and antibiotic resistance.

Key environmental factors include: 1) Climate: Indonesia's tropical climate, with its high temperatures and humidity, promotes the growth of Shigella sp., as warm water accelerates bacterial reproduction and increases the likelihood of resistance mutations; 2) Water pH: *Shigella* sp. thrives in water with a neutral to slightly alkaline pH (6.5-7.5). Deviations in pH outside this range can limit bacterial growth, but favorable pH conditions enhance survival and resistance; 3) Temperature: Consistently high water temperatures (above 25°C) in tropical regions support bacterial survival and resistance gene expression, allowing *Shigella* sp. to dominate, particularly in polluted water bodies; 4) Rainfall intensity: heavy rainfall, typical during monsoon seasons, increases surface runoff from urban and rural areas, carrying contaminants such as untreated sewage, agricultural waste, and industrial effluents. This enhances the bacterial load, including antibiotic-resistant strains [9,15,16,17]. In addition to environmental factors, human activities significantly influence *Shigella* sp. contamination and resistance patterns: 1) Urban wastewater disposal: Untreated sewage from urban areas, containing human pathogens and antibiotics, introduces contaminants into rivers, fostering bacterial resistance; 2) Industrial pollution: Effluents from industries, including food processing and pharmaceutical plants, contain antibiotics and pollutants, creating conditions that select for resistant *Shigella* sp.; 3) Agricultural runoff: Antibiotics used in livestock farming enter rivers through runoff, encouraging the development of resistant bacterial strains. Fertilizers and pesticides further disrupt the microbial balance, favoring resistant bacteria; 4) Sanitation and hygiene Practices: Poor sanitation infrastructure, including open defecation and improper waste disposal, increases the bacterial load in rivers, enhancing the risk of antibiotic resistance; 5) Antibiotic misuse: The overuse of antibiotics in healthcare and agriculture, combined with easy access to antibiotics in urban areas, contributes to the spread of resistance in *Shigella* sp. This combination of geographical and human factors accelerates the development and spread of antibiotic-resistant bacteria in Indonesian rivers, posing significant public health risks [18–23].

Shigella sp. is generally unstable in the aquatic environment, but if found, it indicates pollution from human feces [24]. A lack of understanding and awareness regarding the use of antibiotics makes people misuse antibiotics, which play an essential role in antibiotic resistance [25]. The inappropriate use of antibiotics during the pandemic can also worsen antibiotic resistance in Indonesia [26]. Based on observations around the Bedadung River, several people from various sectors carry out anthropogenic activities. Antibiotics in the livestock sector are not only used to treat microbial infections. However, they are also used as antibiotic growth promoters that aim to accelerate growth and increase the appetite of livestock, such as broiler chickens [27]. Antibiotics that are not adequately metabolized by livestock will produce metabolites excreted in the urine and feces. Antibiotic residues often found containing antibiotic-resistance genes in livestock wastewater are tetracyclines, aminoglycosides, sulfonamides, and macrolides [28]. Increasing temperatures due to climate change can disrupt the animal immune system in livestock and fisheries, making animals susceptible to disease [29].

Antibiotics in fisheries are also used to induce growth and prevent infectious diseases. Chloramphenicol is the most commonly found antibiotic residue in fishery wastewater [26]. Industrial effluents can contain heavy metals like copper, zinc, nickel, arsenic, and mercury. Heavy metal components can persist in the environment and exert long-term selective pressure that aids in forming ARGs. Antibiotic resistance due to heavy metal contamination can occur through co-resistance, cross-resistance, and co-regulation mechanisms. Co-resistance mechanism is the antibiotic and heavy metal resistance mechanism that coincides due to the same encoded mobile genetic elements, such as plasmids, transposons, and integrins. Cross-resistance and co-regulation mechanisms occur due to the activation of the efflux pump to eliminate antibiotics contained in bacterial cells. Activation of efflux pumps in the co-regulation mechanism is caused by the initiation of transcription and translation processes in DNA replication, which causes bacteria to experience metabolic stress. Heavy metals and antibiotic residues from iron industry waste will encourage bacteria to produce ARGs. Antibiotics that have recorded resistance and are associated with heavy metal waste contamination are penicillins and cephalosporins [30,31].

Inappropriate land use can lead to erosion, soil quality, and structural degradation. Soil erosion rates are influenced by climate and rainfall intensity. When rainfall intensity is high, the volume of water in the environment will increase, risking soil erosion and flooding. Anthropogenic activities carried out by the community, such as bathing, washing clothes, and defecation in the river, will produce a variety of waste pollutants. Waste in the form of chemicals and piles of garbage can carry antibiotic residues, threatening public health. The accumulation of waste in the Bedadung River can increase the pH of river water, which can become a reservoir for Shigella sp. and increase the solubility of antibiotic residues in river water. The bacterial defense against high concentrations of antibiotic residues is to produce ARGs. These components can survive or thrive in the environment by HGT to other bacteria through various media, such as water, soil, air, food, and living [32].

Some antibiotic residues, such as tetracyclines, sulfonamides, and fluoroquinolones, have a chemical structure in the form of a heterocyclic ring stronger than other antibiotics, making it difficult to eliminate through the biodegradation process. As a result, these antibiotic residues will be easily detected in the environment, especially in aquatic environments [28,33]. These conditions may interfere with the river's ability to biodegrade polluting effluents. Even though it comes from several different areas, the polluting waste will collect into one flow that empties the downstream of the river. Then empties into the Bedadung River downstream. This is in line with the results of research by Pangastuti et al. (2022), which show that the value of the Bedadung River pollution load will increase when heading downstream. If the water pollution load of the Bedadung River is excessive, the river will be polluted and cannot be adequately used [34].

This study has notable social implications, particularly for public health, environmental justice, policy development, community empowerment, and local economies. Identifying antibiotic-resistant *Shigella* sp. in the Bedadung River highlights serious health risks, especially for rural communities with limited clean water and healthcare access. The research can promote public health campaigns and equitable environmental policies, addressing the disproportionate impact on rural areas. It may also influence stricter regulations on waste management and agricultural practices. Community engagement in water management could foster responsible behaviors, while addressing contamination is critical for protecting livelihoods, especially in agriculture and fisheries. Overall, the study aims to improve health outcomes and environmental sustainability.

The limitation of this study is that the river water isolates used in rural and urban areas only use the same river, the Bedadung River. Waste originating from both areas has the potential to collect into the same flow and empty downstream of the Bedadung River. In addition, this study does not further examine the types of *Shigella* sp. bacteria species, metagenomic analysis, and antibiotic resistance genes contained in Shigella sp. bacteria isolated from Bedadung River water in Jember Regency. In conclusion, this study found no significant relationship between rural and urban settlement with the *Shigella* sp. resistance pattern isolated from the Bedadung River in Jember, Indonesia. Further studies on the antimicrobial resistance risk factor are suggested. Therefore, other studies can compare antibiotic resistance patterns with isolates of two rivers in rural and urban areas. In addition, further research is needed relating to the type of *Shigella* sp. bacteria species, metagenomic analysis, and antibiotic resistance genes contained in *Shigella* sp. bacteria isolated from Bedadung River water in Jember Regency.

CONCLUSION

This study found no significant correlation between settlement location (rural or urban) and the resistance pattern of *Shigella* sp. isolated from the Bedadung River in Jember Regency. *Shigella* sp. contamination was equally prevalent in the river's rural and urban sections, indicating that bacterial load and resistance patterns are consistent across these areas. However, a key limitation of the study is the use of water isolates from only one river system, where waste from both rural and urban areas converges downstream, potentially masking location-specific differences in contamination sources. Another limitation is the lack of detailed analysis of the specific species of *Shigella* sp., the absence of metagenomic analysis, and the identification of antibiotic-resistance genes within the isolates. Addressing these gaps could provide deeper insights into the bacterial diversity and the mechanisms driving antibiotic resistance in the river system.

For future studies, it is recommended to: 1) Compare multiple river systems: Investigate resistance patterns using water isolates from different rivers that pass through distinct rural and urban areas, rather than focusing on a single river, to assess better how geographic and environmental factors influence contamination and resistance; 2) Conduct species-level identification: Differentiate between species of S*higella* sp. in future research, which can help identify whether certain species are more prevalent or resistant in specific areas; 3) Utilize metagenomic analysis: Employ metagenomic approaches to explore the broader bacterial community in the river system and uncover the full range of antibiotic resistance genes, providing a more comprehensive understanding of resistance mechanisms in contaminated water bodies. By expanding the scope of the study to include these elements, future research can offer more detailed insights into the factors contributing to antibiotic resistance and provide targeted solutions for mitigating public health risks.

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