Antibiogram Fingerprints of Bacteria Isolated from River Water Near a Hospital in Malang

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Abstract. The focus on the environment as one of the dimensions of the transmission and acquisition of antimicrobial-resistant bacteria has been increasing since the campaign to combat resistance adopted the One Health framework (human-animal-environment). Hospitals, as the highest users of antibiotics, often dispose of their waste into rivers, which has the potential to spread antibiotic resistance to the environment. This study examines the susceptibility profile of 18 bacteria isolated from river water near a hospital against 6 types of antibiotics. Based on the resistance patterns, two unique patterns of MARPs (Multiple Antibiotic Resistance Profiles) to antibiotics were observed: one isolate showed resistance to 4 antibiotics with a MARI (Multiple Antibiotic Resistance Index) value of 0.6, and another showed resistance to 3 antibiotics with a MARI value of 0.5.

1 Introduction

The one of the biggest challenges in the field of public health in the world is the high increase in the number and types of microbial resistance (AMR) [1]. The use of antimicrobials in various health facilities, both for humans and animals, contributes to the extensive widespread of antimicrobial resistant bacteria which not only impact humans and animals but also the environment such as air and soil [2, 4]. As a result, the probability of being exposed to these bacteria increases sharply even outside health facilities. For instance, humans can be exposed during preparation or when consuming meat, vegetables, fruit, or contact and accidentally swallow water contaminated with antimicrobial resistant bacteria [3]. Since it was first discovered that bacteria can become resistant to antibiotics, this could have a critical impact on irrigation water and agriculture which is animals and humans have used in their daily lives. The presence of antibiotics and other factors that contribute to resistance may increase the appearance of antibiotic resistance genes (ARGs) in the genetic designs of bacteria found naturally in rivers, both in their chromosomes and in mobile genetic elements.

The dissemination of resistant bacteria through direct or indirect exposure could endanger human health. The direct impact of exposure to antimicrobial resistant pathogens is the difficulty of treating the result of infections. The impact of indirect exposure is from relatively inoffensive resistant bacteria such as commensal bacteria that colonize the

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digestive tract, skin and mucosa which results in asymptomatic carriers of the resistant bacteria. This condition is not without risk, but it can allow the transfer of resistance genes between commensal bacteria and pathogenic bacteria which can be dangerous for people who are susceptible to infection, such as the elderly, individuals with compromised immune systems, and individuals with underlying diseases [5].

Since there was a global and national campaign to overcome antimicrobial resistance that carries the One Health (human-animal-environment) framework, the focus of attention on the environment as one of the dimensions of transmission and acquisition of antimicrobial resistant bacteria has increased [2]. Water is considered the most important medium for the dissemination of resistant bacteria in the environment due to its interaction with microbes associated with humans. The most significant source of pollution of aquatic ecosystems by antimicrobial residues and resistant bacteria comes from hospitals [6, 7].

Hospitals are an ecological niche for antimicrobial-resistant bacteria [8]. Every hospital must have a good waste management system so that it can guarantee that waste is safe from contaminants before being discharged into the environment. However, hospitals often have problems in managing this waste. Poor waste management can be dangerous for the environment. Moreover, in Malang City there are several hospitals located near rivers. This condition can allow resistant bacteria to be excreted into surrounding rivers, thereby accelerating their spread into the environment. Therefore, it is necessary to carry out research to detect and identify antimicrobial resistant bacteria in the aquatic environment close to hospitals in Malang City. The results of this identification can later be used as a basis for tracing the most likely sources of contaminants as well as possible efforts to overcome the further spread of resistant bacteria.

2 Material and Method

2.1 Collection of Samples

In this study, 18 bacterial isolates from 4 genera/species suspected to be *Staphylococcus aureus* (isolate number: 6, 8, 29, 31, 32, 44, 45), *Escherichia coli* (isolate number: 14, 17, 24, 26, 27, 42, 43, 48), *Pseudomonas sp.* (isolate number: 12 and 41), and *Shigella sp.* (isolate number: 4) were used. These were obtained from morphological and biochemical identification of water samples taken from 3 locations: the drainage pipes of the hospital's liquid waste, and before and after these drainage pipes (Lat -7.930°, Long 112.598°). The proximity of the location between the river and the hospital was the basis for conducting research on suspicion that consumption of antibiotics and the irrigation of hospital waste into the river had an impact on bacterial mutations that cause antibiotic resistance.

2.2 Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out using the standard Kirby-Bauer disc diffusion method on Mueller Hinton Agar (MHA) media referring to Disk Diffusion Test as approved by the Clinical Laboratory Standards Institute (CLSI). Inoculum preparation was carried out by mixing new 24 hours old colonies into 1 mL of sterile salt solution equivalent to 0.5 McFarland and then inoculating on agar medium.

Testing was carried out on eighteen isolates with antibiotics belonging to six classes of antibiotics which is often dispensed on these isolates were used for susceptibility testing and include penicillin (penicillinase), gentamycine (aminoglycoside), tetracycline (tetracycline), Ciprofloxacin (fluoroquinolones), chloramphenicol (phenicol), and erythromycin (macrolide) were used to view the bacterial susceptibility profile. The inoculation results were then incubated at 37 °C for 18-24 hours. The diameter of the inhibition zone formed is calculated and classified based on CLSI 2020, criteria the values were interpreted as resistance (R), intermediate (I), or susceptible (S), and for those that are not listed for the genus/species in question, we classify them as an unidentified group.

2.3 Evaluation of Patterns of Multiple Antibiotic Resistance Index of Isolates (MARI)

In evaluating multiple antibiotic resistance index patterns by considering multidrug isolates generated using mathematical equations adapted from [8,9], which are formulated in Equation 1.

$$\text{MAR index} = a/b \quad (1)$$

Symbol ‘a’ refers to the number of antibiotics to which isolates exhibit resistance, and the symbol ‘b’ refers to the number of antibiotics against which each isolate was tested.

3 Results and Discussion

3.1 Evaluation of antibiotic susceptibility testing

The susceptibility profile of eighteen isolates to test antibiotics is displayed in Fig. 1. The findings showed that there was a significant incidence of resistance against penicillin (61%). But there was also evidence of a high susceptibility to gentamicin (100%), chloramphenicol (78%), tetracycline (67%), and ciprofloxacin (61%). While susceptibility to erythromycin was detected at only 22%, with a resistance rate of 11%. This occurred because most of the isolates we used were Gram-negative bacteria, resulting in there being no cut-off for erythromycin in the CLSI 2020 guidelines, which led to 56% falling into the unidentified group.

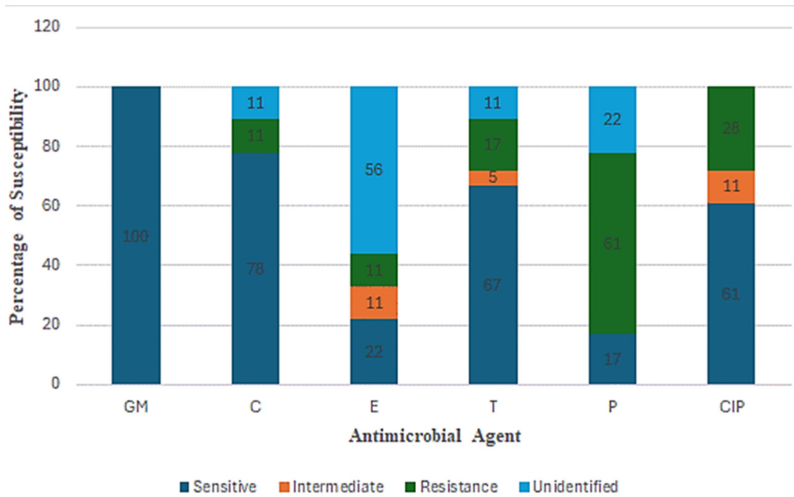

Fig. 1. Antibiotic susceptibility profile of 18 isolates. GM: Gentamicin, C: Chloramphenicol, E: Erythromycin, T: Tetracycline, P: Penicillin, and CIP: Ciprofloxacin

The previous study on antibiotic susceptibility testing of *E. coli* isolates obtained from surface water samples in the city of Palembang revealed high resistance to tetracycline (71%), whereas resistance to ciprofloxacin was observed at only 7%, and to gentamicin and chloramphenicol at 4% each [15]. In another study, the disk diffusion antibiogram of Pseudomonas isolated from two locations revealed complete resistance to penicillin and complete susceptibility to ciprofloxacin and gentamicin, while resistance to tetracycline varied in percentage [14]

3.2 The Pattern of Antibiotic Resistance Phenotype

The heatmap in Fig. 2. illustrates the phenotypic antibiotic resistance patterns of each isolate, which indicates the detectable antibiotic resistance characteristic that each isolate possesses. This provides an indication of the genetic features that influence each strain. Referring to the result, from water sampling of river near hospitals exhibited 2 patterns of MARPs to antibiotics uniquely, only one isolate that showed resistance to 4 antibiotics that have value 0.6 of MARI and one another showed resistance to 3 antibiotics that have value 0,5 of MARI. The value of MARI shown consideration the degree of the river contamination by antibiotic that may be serious problem for human health [11]. A value more than 0.2 indicate a high risk exposed by antibiotics and the value that lower than 0.2 indicated low exposured by antibiotics [12]. In this study the MARI result of MDR tests shown in Table. 1.

Isolates from water sampling have MARI value above 0.2 that mean permissible to occupy the benchmark for MAR. This shows that bacteria have broader resistance to the antibiotics used in the test and need serious attention because antibiotic resistance can make treating infections more difficult and require intensive treatment [10]. The environment near to the hospital may be a major role factor in the implications of bacterial exposed by antibiotics, especially in the context of water, a high MARI can indicate fecal contamination or an abnormal environment. The impact of increasing the resistance of a bacteria in river affects the risk of public health, where the water is commonly used to support community activities, threaten food safety and increase the risk of spreading resistant bacterial infections.

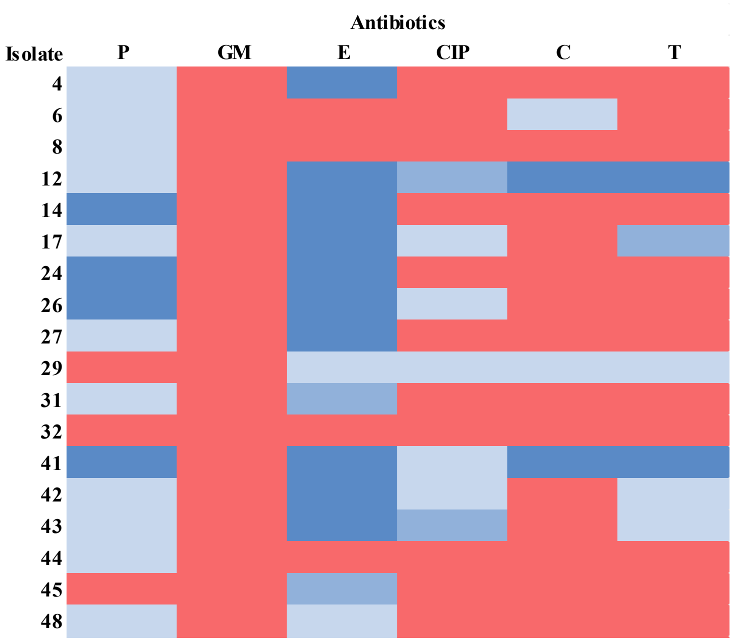

Fig. 2 Heatmap showing phenotypic antibiotic resistance pattern of each isolate. The colors of : refers to sensitive (S) of antibiotic, : refers to resistance (R) of antibiotic, : refers to intermediate (I) of antibiotic, and : refers to unidentified of antibiotic.

Table 1. Patterns of MAR phenotypes and MAR Index of bacteria isolated from irrigation water samples near hospitals.

MAR Pheynotypes	No. of Antibiotics	No. Observed	MARI
P-CIP-TE	3	1	0.5
CIP-TE-E-C	4	1	0.6

In previous study of MAR on antibiotic testing of *E. coli* that isolate obtained from Tyhume River and Tsomo River reveals 13 isolated that have MAR index above 0.2 which is resistance to chloramphenicol, tetracycline, erythromycin, gentamicin, and ciprofloxacin [13]. It is important to know in this study, prevalence of susceptibility shown that isolate is exposed to chloramphenicol, erythromycin, tetracycline, penicillin, and ciprofloxacin. Even though the percentage of susceptible is higher except in ciprofloxacin. This gives evidence that there are five existence of antibiotics uses except gentamicin that settles or is present in the river surface and ciprofloxacin have higher prevalence percentage of antibiotic compared to other four antibiotics.

One of the key issues is the disposal of wastewater, as rivers are the primary recipients of waste from various disposal activities. Unfortunately, many antimicrobial agents are not fully metabolized in the body and are subsequently released directly into hospital sewage systems or municipal wastewater and in this case the the wastewater of hospital as major effect to environtment of water rivers.

4 Conclusion

Based on the results of this study, it can be concluded that most isolates still show a relatively high level of susceptibility to the antibiotics used, although 2 MARP patterns were also found. We recommend increasing the sample size of the study to include various types of bacteria. Additionally, we suggest selecting antibiotic types in accordance with the latest CLSI guidelines to provide a more meaningful picture of antibiotic resistance status in the river environment.

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