Antibiotic Resistant Bacteria from Sediment of Coastal Water of Pahang Malaysia

Nurhazlin Ab Rahman, Ahmed Jalal Khan Chowdhury, and Zaima Azira Zainal Abidin

Abstract—This study was conducted to determine the presence of antibiotic resistance bacteria in sediment of coastal water of Pahang, Malaysia. A total of 64 bacteria were isolated and tested against 10 different antibiotics (Vancomycin, Tetracycline, Chloramphenicol, Streptomycin, Gentamicin, Penicillin G, Ampicillin, Erythromycin, Rifampicin and Polymyxin B). The greatest frequencies of resistance in bacteria were found in Polymyxin B (94%), Streptomycin (72%) and Penicillin G (50%) in Balok whilst, the high resistance of bacteria in Teluk Chempedak existed in Polymyxin B (100%), Erythromycin (63%) and Streptomycin (43%). Approximately 91% of Balok isolates and 87% of Teluk Chempedak isolates showed Multiple Antibiotic Resistance (MAR) index value higher than 0.2 (≥0.2) which suggest that there is a high-risk of antibiotics contamination in the area. Hence, findings from this preliminary study revealed the presence of antibiotic resistance bacterial strain in coastal water of Pahang, Malaysia and this may pose a potential public health hazard.

Keywords—Antibiotic resistance bacteria, sediment, coastal water, Pahang.

I. INTRODUCTION

The discovery of antibiotics was undoubtedly one of the greatest achievements of the twentieth century. They have microcidal or microstatic activity which functions to disrupt the microbial metabolism by a variety mechanism. In recent decades, antibiotics have been intensively and widely used in humans and animals, and as growth promoters in agriculture and aquaculture thus increasing the proportion of antibiotic resistant bacteria in various environments [1].

These chemical compounds received little attention as pollutants in the aquatic environment [2]. Apart from chemical pollution caused by the antibiotics, the elevated of concentration of antibiotics in the aquatic environment may also accelerate the development of antibiotic resistance genes (ARGs) and the emergence of antibiotic resistance bacteria [3] which shade risks to humans’ health and disturb the aquatic ecosystems. Bacteria resistant to antibiotic are commonly discovered in clinical and veterinary areas but it is not well characterized in aquatic environments [4]. The resistance to antibiotics has become a severe issue in human health and to the ecosystem function. Many antibiotics persist in the sediment and organisms in the aquatic environment for several months following administration [5]. Their residues may affect the biological action on the microbes if they continually emitted to the environment [6].

Coastal environment plays an important role as habitat to a number of plants and animals which serve as breeding and nursery grounds, shelters, sources of food for various marine lives [7]. Recently, coastal waters are facing a wide variety of stressor affecting both the ecosystem and human health caused by domestic wastewater treatment and disposal practices which led to introduction of high levels of nutrients and enteric pathogens. Besides that, the release of antibiotics from hospital wastewater, development of recreational area and discharge of effluent and wastewater from resorts and restaurant along the coastal area also contribute serious damages to the coastal environments.

Currently, several studies relating to the existence of antibiotic resistance bacteria in sediment of coastal waters have been done [4], [8], [9], [10], however studies conducted on antibiotics resistance bacteria in sediment of coastal water in Malaysia are still lacking. Thus, this study presents a preliminary report on the occurrence of antibiotic resistance in bacteria in coastal water of Pahang, Malaysia.

III. MATERIALS AND METHODS

A. Study areas and samples collection

Pahang is the largest state in Peninsular Malaysia and is situated in the eastern coastal region (Figure 1). The sediment samples were collected from the study areas in Figure 1: Teluk Chempedak and Balok (15km between each location) by using Eckman grab. The sampling was carried out in triplicate at each location with 35m distance of each point.

Fig. 1 Locations of sampling area
(A: Balok, B: Teluk Chempedak)
Each point was determined by Geographical Positioning System (GPS) coordinate (Table 1). The sediment samples collected were transferred into sterile polyethylene bags and processed immediately. At each location, the physicochemical parameters such as temperature and pH were recorded by using YSI multi-probe hydrolab.

### TABLE I

<table>
<thead>
<tr>
<th>Coordinate</th>
<th>Balok</th>
<th>Teluk Chempedak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point 1</td>
<td>N 03°55.768 E 103°23.395</td>
<td>N 03°48.770 E 103°22.633</td>
</tr>
<tr>
<td>Point 2</td>
<td>N 03°56.115 E 103°23.536</td>
<td>N 03°48.650 E 103°22.573</td>
</tr>
<tr>
<td>Point 3</td>
<td>N 03°56.397 E 103°23.660</td>
<td>N 03°48.465 E 103°22.547</td>
</tr>
</tbody>
</table>

### B. Isolation of Bacteria

Bacteria from sediment samples were isolated by spread plate technique. One gram of the sediment samples were mixed with 10 ml autoclaved saline. The homogenized samples were diluted 1:10 v/v with saline and followed by serial dilutions (10^-2 to 10^-6). About 100 μl of dilutions 10^-3 to 10^-6 were plated on nutrient agar and incubated for 36hr at 37°C. After incubation, the representative colonies were chosen randomly from the plates and purified on nutrient slant agar medium for further study. Gram-positive and Gram-negative bacteria were identified using gram stain.

### C. Antibiotic resistant test

Antibiotic resistance bacteria were determined using disc diffusion method following the procedures of standardize agar diffusion method by National Committee for Clinical Laboratory Standards (NCCLS) for antibiotic susceptibility tests [11]. The isolates were inoculated in Mueller-Hinton Broth and incubated for 18hr at 37°C. The turbidity of the bacterial suspension was adjusted and compared with 0.5 Mc Farland Standard using spectrophotometer at 625 nm. The amount of the bacteria was equivalent to 1.5 x 10^8 cfu/ml. About 100 μl of bacterial suspension was spread on agar plate and left to dry before placing the antibiotic disc on the surface of the plate. All the samples were treated with 10 different antibiotics to test their susceptibility towards the antibiotics. The antibiotics used were namely Tetracycline (30 μg), Rifampicin (30 μg), Streptomycin (10 μg), Vancomycin (30 μg), Penicillin G (10 units), Ampicillin (10 μg), Chloramphenicol (30 μg), Gentamycin (10 μg), Erythromycin (15 μg) and Polymyxin B (300 units) which are available from Oxoid in disc form. The results were documented by measuring the inhibition zone of the colonies that formed on the plates after 24hr of incubation at 37°C. The diameter of inhibition zones was measured to the nearest mm and the isolates are classified as resistant (R ≤ 16mm) [11].

### D. Determination of MAR index

The Multiple Index Resistance (MAR) index values of each isolates were determined by dividing the number of antibiotics to which isolate is resistant by the total of antibiotics tested [12], [13]:

\[
\text{MAR index value} = \frac{a}{b}
\]

Where ‘a’ represents the number of antibiotics that isolate bacteria show resistant to and ‘b’ represents the total number of antibiotics the isolate bacteria show non-resistant. If the MAR index value of isolate is ≥ 0.2, it indicates high risk of contamination of several antibiotics used in the environment of the isolate originated [4], [10].

### III. RESULTS

The physicochemical parameters such as temperature and pH of the study area were determined using YSI multiprobe hydrolab. The temperature in both locations are ranged around 27 - 28°C and the pH is ranged 8.0 - 8.2 which is the normal condition for seawater.

There were total of 64 bacteria (32 isolates each location) were randomly selected and examined for antibiotic resistance test. The results (Figure 2) from the test showed that the greatest frequencies of resistance in bacteria were found in Polymyxin B (94%), Streptomycin (72%) and Penicillin G (50%) in Balok whilst, the high resistance of bacteria against the antibiotic in Teluk Chempedak existed in Polymyxin B (100%), Erythromycin (63%) and Streptomycin (43%). Meanwhile, the resistance of the bacteria to Chloramphenicol and Rifampicin were less common in both locations (Figure 2) as both antibiotics showed low percentages of resistance isolates.

![Fig. 2 Percentage of bacterial resistance to different antibiotics used in Teluk Chempedak (%)](image-url)
The multiple antibiotics resistance (MAR) index value of each isolate was calculated to determine the resistance of bacteria to multiple antibiotics (Figure 3 and Figure 4). From the findings, multiple antibiotic resistances were observed in isolates from both locations. In Balok, most isolates showed resistance to 4 antibiotics compared to Teluk Chempedak, where most of the isolates showed resistance in 2 antibiotics (Figure 3 and Figure 4).

Majority of the bacteria isolated from both locations were Gram negative bacteria. From the findings, the Gram negative isolates at both locations had a higher frequency of MAR index value ≥0.2 compared to the Gram positive isolates (Table 2). The results also revealed that some of the Gram negative isolates showed high antibiotic resistance activity up to 8 antibiotics compared to the Gram positive isolates. About 91% of Balok isolates and 87% of Teluk Chempedak isolates showed MAR index value higher than 0.2 (≥0.2) which suggest that there are high-risk of antibiotics contamination in both areas (Figure 5). Hence, from the results, it indicates that Balok was probably more exposed to multiple antibiotics contaminants compared to Teluk Chempedak.

### IV. DISCUSSIONS

Coastal areas are known to serve as “recipient” for the most forms of anthropogenic contaminants including the antibiotics. Wide and unnecessary usage of antibiotics poses risks to the aquatic ecosystems and human health due to the ability of bacteria to develop the resistance genes and transfer the resistance genes into other bacteria in the environment including the transfer between the Gram positive and Gram negative bacteria by plasmids [2], [12].

The resistance in bacteria can be spread through the population in three different ways. First, the resistance can be transferred by the transfer of bacteria between people, second is by the transfer of resistance genes or plasmid between the...
bacteria and third by the transfer of resistance genes between the genetic elements within the bacteria (transposons) [14]. The differences in percentage of bacterial resistance to various antibiotics in this study could reflect the antibiotic contamination in the area and could be considered as a bioindicator to initiate the monitoring programs for antibiotic pollution [15].

In this study, majority of the isolates were found to be resistant to polymyxin B. It is assumed that polymyxin B containing agents are widely used in Kuantan, which may lead to the development of resistant bacteria to polymyxin B. This antibiotic is commercially available as polymyxin B sulfate which clinically used as a topical medication (skin, ears and eyes), parenteral (intravenous and intrathecal administration) and for respiratory tract disease [16], [17]. Polymyxin B is commercially used in ointments to treat soft tissue infections which known as Neosporin [18].

Meanwhile, low percentage of resistance against chloramphenicol were exhibited in most of the isolates suggests that this antibiotic is not common in Kuantan. This finding is also in coherent with previous studies conducted in Malaysia which also showed low percentage of bacteria resistance to chloramphenicol [8], [9].

Furthermore, majority of tested isolates were Gram negative bacteria and they displayed stronger resistance towards the antibiotics tested than Gram positive bacteria. Most of Gram negative bacteria are pathogenic to human. Previous study reported that plasmids encoded with antibiotic resistance genes were transferred between the pathogenic and non-pathogenic Gram negative bacteria in the environment which presumed the high resistance activity towards the antibiotics [19]. Besides, these findings also implied that the pathogenic bacteria had numerous opportunities to become resistant to antibiotics, thus making it more difficult to prevent bacterial disease.

Apart from that, due to indiscriminate use of antibiotics the bacteria might have developed resistance towards several antibiotics. The presence of antimicrobial agents at low concentration through leaching or continued usage may lead to the development of drug-resistant strains and multiple antibiotic resistances in bacteria, which result in the transfer of resistance to pathogenic bacteria and reduced efficacy of antibiotic treatment for human and animal diseases [20]. The multiple resistance or ‘multi-resistance’ is considered to be the key indicator of problematic bacterial strains because it undermines the empirical treatment regimens which delay the administration of the appropriate antibiotic therapy, and it also reduces the options of treatments that are appropriate [21]. In this study, both locations (Balok and Teluk Chempedak) exhibited high percentage of MAR index value ≥0.2 that suggested a variety of antibiotics are being disposed at these areas. The emission of these antibiotics contaminants might be from the human activities on the beach or water. Besides that, the discharge of effluent and waste waters from the resorts and restaurant which located along the coast of Balok and Teluk Chempedak might also be the source of the antibiotics contaminations of the areas.

Marine recreational waters and sands had received domestic sewage which may contribute to the establishment of distribution routes by microbiorganism that carrying antimicrobial resistance gene [22], [23]. Furthermore, the existence of antibiotic resistant gene in bacteria may be influenced by human urban and agricultural activities, including sewage discharge, animal husbandry, and fish breeding, as well as natural environmental changes [24]. Human intestinal bacteria are reservoirs for antibiotic resistance genes. They not only exchange resistance genes among themselves but might also interact with bacteria that are passing through the colon, causing these bacteria to acquire and transmit the antibiotic resistance genes to the environment through fecal excretion [25]. These factors not only lead to the occurrence of antibiotic resistant bacteria carrying antibiotic resistant genes encoding resistance to some kinds of antibiotics directly, but also change the water parameters to influence the growth conditions of microorganisms, resulting in changes in the structure of the microbial community. Besides, the key factor in the development of antibiotic resistance bacteria is that bacteria have the ability to adapt quickly to the new changes in the environment conditions and survive [26]. Therefore, a single genetic mutation in bacteria such as development of resistance genes can greatly affect their ability to cause disease. As the bacteria could reproduce by dividing every few hours and evolve rapidly, this could be the reason of the spreading of resistance genes in the bacteria population in the area. Antibiotic resistance in bacteria is considered to be major problem because many disease causing bacteria are becoming more resistant to commonly use antibiotics. The overuse of antibiotics and the lack of monitoring waste water discharge into the environment might leads to the development of antibiotic resistance bacteria.

V. CONCLUSION

This study demonstrates the incidence of antibiotic contamination in Pahang coastal waters as indicated by the presence of antibiotic resistance bacteria in the marine sediment. Moreover, majority of the bacteria exhibited multiple antibiotic resistances which further put Pahang coastal water at risk of being more exposed to severe antibiotic contamination if no measures or actions are taken to circumvent the problem. Findings from this study could provide further understanding on the status of antibiotic contamination in Pahang coastal water. The development of antibiotic resistance in bacteria are the issues that need to be investigated in greater depth, suggesting the importance of wise use of antibiotics and their risk to human health and the environment in general. Thus an urgent need is required to monitor antibiotic drugs that are used in order to reduce the risk of antibiotic residues contamination and bacterial resistance at the coastal water of Pahang, Malaysia. This study could also be used as a basis for more extensive study which
can provide further understanding on the status of antibiotic contamination in Pahang coastal water.

VI. ACKNOWLEDGMENT

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REFERENCES


