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Prevalence and Diversity of Antibiotic Resistance Heterotrophic Bacteria Found Along the Bintulu Rivers, Sarawak

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ABSTRACT

The study aims (1) to isolate and characterise the heterotrophic bacteria from different rivers in Bintulu, Sarawak, (2) to investigate the hygienic condition of the rivers through faecal coliforms and (3) to determine the antibiotic resistance among the heterotrophic bacteria isolates. A total of 100 heterotrophic bacteria strains were identified from rivers of Bintulu, Sarawak. The characterisation of bacteria was performed using $(GTG)_5$ fingerprinting to investigate their genetic distribution diversity and 16S rRNA gene sequencing. Microbiological variables tested including total viable count, coliform count, *Escherichia coli* confirmation test. Antibiotic susceptibility test was performed against 10 antibiotics. Sample collected from Sungai Teknik showed higher mean bacterial population size (log 4.48 ± 0.00 CFU/mL). Sungai Sibiu, Waterfront and Sungai Plan revealed the highest most probable number (MPN) index (>1,600 per mL). Each dendrogram showed 3 to 7 clusters of bacteria groups confirmed as *Enterobacter* spp. (40%), *Acinetobacter* spp. (13%), *Bacillus* spp. (13%), *Klebsiella* spp. (13%), *Staphylococcus* spp. (7%), *Chromobacterium* spp. (7%) and *Citrobacter* spp. (7%). All the heterotrophic bacteria isolated showed high resistance against ciprofloxacin (63.70 ± 33.40%), piperacillin (58.10 ± 31.37%), aztreonam (48.40 ± 30.95%) and more susceptible to tetracycline (3.30 ± 10.44%). Our findings highlight the multiple antibiotics resistance and microbiological analysis of heterotrophic bacteria found in polluted river water. The preservation of the river water is vital as hydrologic purposes, sustain the microbial composition, ecological integrity of the river.

Keywords: Antibiotic resistance, bacteria, coliform, heterotrophic, rivers

INTRODUCTION

River microbial diversity is important especially as drivers in our aquatic ecosystem functioning. Bacteria act as organic matter decomposer, respiratory mediator, carbon (C) flow mediator (Fabian *et al*., 2017), and as food web structurer (Shurin *et al*., 2012; Trombetta *et al*., 2020) in ecosystem. The nitrogen (N) uptake process of microbial in smaller rivers may also affect the water quality flowing in downstream river (Xia *et al*., 2018). Microbial diversity differs in space and time. The differences are caused by the variation in nutrient availability (Mello *et al*., 2016), organic matter (Maron *et al*., 2018), temperature of the water, hydrological factors, land management purpose (Sui *et al*., 2019), season and environmental variables (Gilbert *et al*., 2011). The global environmental changes (Gilbert *et al*., 2011) and contamination are the major factor known to drive ecosystem processes and give rise to heterogeneity in microbial diversity. Zeglin (2015) reported that bacterial diversity within rivers is strongly affected by the different level of organic matter decomposition across the different rivers. Global warming or climate change create numerous prospective menaces to the operation of the aquatic ecosystems. Zeglin (2015) reveals that these progressive changing factors will directly affect the biological operations of the lotic ecosystems in rivers, streams, or springs. The importance of these microbial role in lotic ecosystem is critical mainly linked to genetic diversity which reveals most information regarding their potential resilience under the global environmental changes. Li *et al*. (2021) explained that the study regarding the microbial diversity in stream and river in lotic ecosystem has been studied, yet a synthesised understanding is still lacking. Studies from rivers may provide more understanding and knowledge

regarding the freshwater bacteria response to the environmental disturbance and their composition, bacterial structure and even the diversity dynamics (Eiler *et al*., 2012).

Healthy aquatic ecosystem is needed to prevent any disease outbreaks. Microbiological assessment is vital to assess the risk of bacteria overgrowth caused by contamination which may have the possibility of spreading pathogens to animals or plants (Leong *et al*., 2018). Enteric pathogens in river cause most of the waterborne illnesses (Pandey *et al*., 2014), thus coliform tests which include total coliform, faecal coliform and *Escherichia coli* confirmation are good indication for evaluating fecal contamination in unsanitary conditions. The assessment of possible anthropogenic antibiotic resistance present in pathogens is crucial due to the abuse usage of clinical antibiotics in soil-dwelling organisms such as bacteria, fungi, and actinomycetes, waste products from treated animals or humans, runoff from agriculture and hatcheries (Djenadi, 2017).

The Bintulu city is in one of the oils and gas industrial centres in Sarawak, east coast of Malaysia. Rivers support many kinds of agricultural activities, aesthetics, navigations and even source for drinking especially for people who live in developing country as in Malaysia. Rivers are commonly used as transportation system during the old civilization, and it is still in used in some parts of Borneo where the road and infrastructure are lacking. Industrial or residential pollution is the cause for the most devastating river pollution, harming the aquatic organisms, land and human where it will be a major problem if the contaminated water was consumed as drinking water. Assessment reports done by Department of Environment Malaysia (DOE, 2018) documented only 24 out of the 117 rivers (20.5%) in whole Malaysia are still clean and not polluted. The river in Malaysia has a biography of catastrophic incidents originated from domestic and industrial pollution in Klang River which further led to flooding in Kuala Lumpur (Fahmi *et al*., 2011), heavy erosion in Perlis River (Samsudin *et al*., 2011), and high organic matter accumulation in Bertam River, Cameron Highlands (Haron *et al*., 2018). Soegianto *et al*. (2020) reported high level of heavy metal found in cockles due to the serious river pollution in our neighbouring country, Indonesia. The author further explained that the consumption of seafood such as fish, prawn and cockles from these polluted rivers may cause harm to human and disturb our food-chain bio magnification. Moreover, pesticide drained from plantation into river is hazardous to all living organisms. It may cause death or chronic long-term illness to humans by affecting the fertility and development of humans.

Therefore, it is critical to investigate on the river microbial diversity in urbanisation rivers. The study aims (1) to isolate and characterise heterotrophic bacteria from different rivers at Bintulu city, Sarawak, Malaysia (2) to investigate the hygienic condition of the rivers through faecal coliforms count and (3) to determine the antibiotic resistance among the heterotrophic bacteria isolates.

MATERIALS AND METHODS

Location of the Study Areas

The study was conducted in 10 different locations of rivers, Bintulu city, Borneo namely Sungai Kirana (03°15’07 N, 113°04’55 E), Sungai Plan (03°16’26 N, 113°05’49 E), Sungai Kemunting (03°10’35 N, 113°03’13 E), Sungai Kemena (03°10’60 N, 113°1’59 E), Sungai Teknik (03°14’41 N, 113°05’40 E), Sungai Sibiu (03°10’00 N, 113°03’00 E), Tanjung Batu (Sing Kwong) (03°12’03 N, 113°02’27 E), Waterfront (03°10’29 N, 113°02’03 E), Jeti Penambang (03°10’08 N, 113°02’34 E) and Sungai TPU UPM (03°12’22 N, 113°05’01 E).

Sample Collection and Processing

Total of 500 mL of water samples were collected, each from different sampling points at 20 cm depth of the flowing river water. The samples were retained at 4 °C for further analysis.

Bacterial Colony Count

The estimation of the colony forming unit (CFU) in water samples were performed as described by Lingoh *et al*. (2020).

Coliform Analysis

Presumptive coliform test: Five tube fermentation analysis was adopted (Leong *et al*., 2018), using Lauryl tryptose broth (Oxoid Ltd., UK). Five broth tube series was applied: five

double-strength broth tubes (first row), and 10 single strength broth tubes (second and third row) with Durham tubes. The bacteria were inoculated with ratio 5:5:5 in 0.1 mL, 1 mL and 10 mL, respectively, followed by incubation at 37 °C. The tubes were checked for 24 and 48 h, respectively. Positive coliform was indicated by the medium colour changed and gas production.

Confirmation test: A loopful of culture from the previous test medium was transferred into brilliant green lactose bile (Oxoid, England) broth containing Durham tubes. Tubes were further incubated for 24 up to 48 h at 37 °C for total coliforms while 44.5 °C for faecal coliform. Any gas production was observed.

Completed test: Confirmation test was done by streaking on EMB agar plate and incubated for 24 h at 37 °C. Colonies showing green metallic sheen were indicated as positive. The most probable number (MPN/100 mL) was recorded.

DNA Extraction

Bacterial DNA was extracted according to Leong *et al*. (2020). Approximately 1.5 mL of overnight bacterial culture grown in Nutrient broth (Merck, Germany) were centrifuged for 5 mins (16770 x g). Supernatant was removed. Total of 500 µL of sterilised distilled water was appended to resuspend the pellet. After that, the suspension was stewed at 100 °C for 10 mins and instantly chilled in ice for 5 mins. The final product was centrifuged for 10 mins (16770 x g) and supernatant was collected.

$(GTG)_5$-PCR

$(GTG)_5$ analysis was implemented as by Sien *et al.* (2013a). A total volume of 25 µL of PCR mixture was prepared consists of 5 µL of 5X Buffer (Promega, USA), 0.8 µL of 25 mM deoxyribonucleotide phosphate (Promega, USA), 3 µL of 25 mM magnesium chloride (Promega, USA), 1 µL of 25 mM $(GTG)_5$ primer (5'-GTGGTGGTGGTGGTG-3') (First Base, Malaysia), 9.9 µL of sterilised distilled water, 0.3 µL of Taq DNA polymerase (Promega, USA) and 5 µL of DNA. The amplification started with initial denaturation at 95 °C (2 mins), next by denaturation, annealing and extension (30 cycles) at 95 °C (1 min), 50 °C (1 min) and 72 °C (1 min), respectively. Final extension was carried out at 72 °C for 5 mins. The amplified PCR product was electrophoresed at 100 V for 45 min on 1.5% (w/v) agarose gel. Post-staining of the agarose gel with 1 µL 10 mg/ mL ethidium bromide was performed before viewing. The RAPDistance package (version 1.04) was applied for scoring profiles retrieved from the gel images.

16S rRNA PCR

Genotypic profiling was performed based on Sien *et al.* (2013b) with some minor modification. Primers were used to amplify the targeted gene: 27F (5'-AGAGTTTGATCCTGGCTAG-3'), 519R (5'-GWATTACCGCGGCKGCTG-3'). The 25 µL mixture made up of 5 µL 5X Taq Green Buffer (Promega, USA), 3 µL 25 mM magnesium chloride (Promega, USA), 1.5 µL 25 mM deoxyribonucleotide phosphate (Promega, USA), 0.5 µL per 20 pmol of each primer (First Base, Malaysia), 4 µL sterile dH_2O, 10 µL DNA template and 0.5 µL of Taq DNA polymerase (Promega, USA). The amplification with 26 cycles begins with initial denaturation stage at 95 °C (10 mins), followed by denaturation at 94 °C (30 secs), annealing stage at 55 °C (1 min), extension stage at 72 °C (1.5 mins) and final extension stage at 72 °C (10 mins). Purification of the amplified PCR product was done by using QIAquick PCR purification kit (Qiagen, Germany) and viewed on 1.0% (w/v) agarose gel at 80 V. Post-staining of the agarose gel with 1 µL 10 mg/ mL ethidium bromide was performed before UV viewing. The purified DNA were sent to for DNA sequencing analysis at Apical Scientific Sdn. Bhd. (Malaysia). The DNA sequencing analysis was done to identify the closest identity matches by comparing with other sequences from data bank attainable in NCBI.

Antibiotic Susceptibility Test

Antibiotics resistance was tested using disc diffusion method in CLSI (2019). Pure bacteria were tested against 10 antibiotics (Oxoid Ltd., England): piperacillin (100 µg), amikacin (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), ceftazidime (30 µg), tetracycline (30 µg), sulphamethoxazole trimethoprim (1.25 µg), streptomycin (10 µg) and aztreonam (30 µg). *Escherichia coli* ATCC 25922 was included as control. The zone diameter for each antibiotic disc was recorded. Result was reported as susceptibility (S), resistant (R) or intermediate (I).

Statistical Analysis

All the data were statistically analysed using SPSS software (version 25). Data subjected to one way ANOVA. All variances between the mean were analysed using Duncan multiple range test (DMR) at 5 % significant level.

RESULTS AND DISCUSSION

Bacterial Count

The mean bacterial colony count ranges from log 3.59 ± 0.06 CFU/ mL to log 4.48 ± 0.00 CFU/ mL as shown in Table 1. The study disclosed that Sungai Teknik showed the highest mean bacterial population size (log 4.48 ± 0.00 CFU/ mL). Table 1 indicates that the mean bacterial colony count was notably different ($p<0.05$) between the two major groups of sampling sites.

The study disclosed that Sungai Teknik showed the highest mean bacterial population size (log 4.48 ± 0.00 CFU/ mL) while Tanjung Batu (SingKwong) showed the lowest mean bacterial population size (3.59 ± 0.06 CFU/ mL). The human activities along the riverbank may affect the diversity of heterotrophic bacteria growth. Most of the rivers in Bintulu city, including Sungai Teknik are flowing near school and housing area. The rivers receive direct flow of the human waste and sewage from the housing drainage system, thus increase possibility of water pollution in those rivers. Sungai Teknik is located near industrial area where all the heavy metals and even harmful untreated discharge may drain into the river, causing pollution. Chen *et al*. (2019) who studied the relationship between pollution and marine sediment microbiome, reported that the diversity of microbe communities may increase and are influenced mainly by human activity and pollution discharge. The lotic ecosystem is vulnerable and subject to change by their surrounding coastal system. The importance of studying microbial diversity is reported as Gillings *et al*. (2015) used environmental microbes as pollution indicators. It acts as an index of comprehensive microbiological assessment in natural and treated water environment.

Coliforms Enumeration

Total coliforms enumeration for the water samples collected from the 10 river samples are shown (Table 1). The presumptive test analysis reviewed the lowest coliform of 23 MPN /100 mL was detected in R8 while the highest coliform exceeded was >1600 MPN/100 mL and it was reported in sites R1, R2 and R3. Site R8 and R5 showed faecal coliform ranged from 23 MPN/100 mL to >1600 MPN/100 mL in site R3. The presence of *E. coli* growth was further assured with the green metallic sheen appearance colonies shown in Table 1.

Coliform counts are another microbiological assessment which give an established indication of the unpolluted governance of a water supply. The coliform count assures the hygienic condition of the rivers while antibiotic resistance test assures clinical safety. The high coliform counts reported in the study indicated the occurrence of faecal contamination (Sanders *et al*., 2013). World Health Organization (WHO) standard limit of coliform count for drinking water is 3 coliform/100 mL (WHO, 1998). The WHO defines safe drinking water source such as water from borehole, dam, river, stream and hand-dug well as low risk: 1 – 10 MPN/100 mL; medium risk: 11 – 100 MPN/100 mL; and high risk more than 100 MPN/100 mL (Odonkor & Mahami, 2020). None of the sites complied with the coliform standard stated in WHO regulation. Three of the samples collected from the rivers reported ≥1600 MPN/ 100 mL. The outcome of the study was in concurrence with Sanders *et al*. (2013), and Leong *et al.* (2018), who disclosed high coliform numbers, ranging from 800 to 1600 MPN/100 mL, based on their inspection of river water in Borneo, Nigeria, and California, respectively. However, the coliform results obtained by Odonkor and Mahami (2020) for the river water was lower as compared to the current study, stated as 10 CFU/100 mL for dry season and 20 – 70 CFU/100 mL for raining season in Ghana. Distribution of faecal coliforms and confirmation of *E. coli* are further validated (Table 1). The faecal counts for sites R3, R6 and R10 (≥1600 MPN/100 mL) were high, thus, the likelihood of contamination with pathogen and human or animal litter are excessive. The facts are further assured by Ekhaise and Omoigberale (2011) who reported that *E. coli* accounted for 19% pollution. Besides, fecal contamination, poor sanitation, and poor management conditions have a major influence on *E. coli* population in aquatic ecosystem was proved by Odonkor and Mahami (2020). Whitlock *et al*.

(2002) reported that predominant faecal coliform is found in avian faeces, wild animals, domestic pets and rodents. Besides, coliform bacteria are well documented as the main polluter in developing country. Site R4, R5 and R8 had better water quality if compared with the others. Urbanisation which leads to overpopulated growth (Evans *et al*., 2012) and accumulation of industrial waste discharged from factories are the leading contributor to river pollution worldwide. The exposed of synthetic substances in sewage may interfere the ecosystem and food web, thus leading to high MPN index. Environmental regulation is one apparent policy tool that can be enforced to curb pollution. In addition, the results obtained provide an inspection guideline in monitoring the water sources.

Table 1. Mean heterotrophic bacterial colony count (CFU/mL) and the most probable number (MPN/100 mL) table for (5 tubes methods) presumptive test analysis of the total and faecal coliform bacteria in 10 rivers in Bintulu, Sarawak

Sampling Sites	Mean total of bacterial colony count (log CFU/mL)[1]	Coliform test (five tubes methods)				
		Total coliform bacteria		Faecal coliform bacteria		*Escherichia coli* confirmation test (10 mL, 1 mL and 0.1 mL)[2]
		Positive tubes in sample concentration (10 mL, 1 mL and 0.1 mL)[2]	MPN Index/100 mL	Positive tubes in sample concentration (10 mL, 1 mL and 0.1 mL)[2]	MPN Index/100 mL	
R1	3.68 ± 0.20[a]	5-5-5	>1,600	5-5-0	240	+++
R2	3.74 ± 0.26[a]	5-5-5	>1,600	5-3-3	170	+++
R3	4.20 ± 0.09[b]	5-5-5	>1,600	5-5-5	>1,600	+++
R4	4.24 ± 0.07[b]	5-2-1	70	5-2-1	70	+++
R5	4.48 ± 0.00[b]	5-1-1	50	5-0-0	23	+++
R6	3.80 ± 0.01[a]	5-5-4	1,600	5-5-4	1,600	+++
R7	4.42 ± 0.01[b]	5-5-4	1,600	5-3-3	170	+++
R8	4.25 ± 0.18[b]	5-0-0	23	5-0-0	23	+++
R9	4.29 ± 0.02[b]	5-5-4	1,600	5-5-3	900	+++
R10	3.59 ± 0.06[a]	5-5-4	1,600	5-5-4	1,600	+++

Legends: R1: Sungai Sibiu, R2: Waterfront, R3: Sungai Plan, R4: Sungai Kemena, R5: Sungai Teknik, R6: Jeti Penambang, R7: Sungai Kirana, R8: Sungai TPU UPM, R9: Sungai Kemunting, S10: Tanjung Batu (SingKwong); [1] The means with the same letter superscript are not significantly different at the 5% level; [2] the data represents number of positive growths.

Heterotrophic Bacteria Profiling

Genetic profiling of the heterotrophic bacteria was pre-screened using $(GTG)_5$ and dendograms were constructed (Figure 1). The goal of performing $(GTG)_5$-PCR is to draw an assumption of hereditary closeness among the heterotrophic bacteria isolated from the 10 rivers. Dendrograms shown in Figure 1 illustrating bacterial community similarity based on a single gene sequencing. Dendrograms constructed using UPGMA similarity distance matrices, acquired by unweighted pair group with arithmetic mean, constituting relative abundance data accessed from the T-RFLP screening of the $(GTG)_5$ genes. Most isolates are well resolved from the others. Homologous recombination event occurs in every river. Each dendrogram tree (Figure 1) displayed two to three major clusters and few sub-clusters. Dendrograms constructed for Sungai Kirana and Sungai TPU UPM showed the most clusters (7 clusters), thus it showed high heterotrophic bacteria diversities for both rivers. Bacteria in the same cluster are belong to the same niche. The divergent types of ventures and practices carried out near the riverbank may affect their microbiological content in the water. The bacteria were further identified using 16S rRNA sequencing analysis and confirmed as *Enterobacter* spp. (40%), *Acinetobacter* spp. (13%), *Bacillus* spp. (13%), *Klebsiella* spp. (13%), *Staphylococcus* spp. (7%), *Chromobacterium* spp. (7%) and *Citrobacter* spp. (7%) (Table 2).

Seven major genera of bacteria isolated from the rivers were identified. *Enterobacter* spp. (40%) was predominantly isolated from the rivers. *Enterobacter* spp. and *Klebsiella* spp., belong to family *Enterobacteriaceae*, which are obligate Gram-negative rod shaped, non-spore forming bacteria commonly found in water source. *Enterobacter cloacae* and *Klebsiella pneumoniae* are potential pathogen which cause community-acquired pneumonia especially in

elderly (Paczosa & Mecsas, 2016). The findings are supported by Suzuki *et al.* (2020) who isolated 11 *E. coli*, 15 *Enterobacter* spp. and 14 *Klebsiella* spp. from hospital wastewater and rivers in the Philippines. Similar finding was reported by Riedel *et al.* (2019) who isolated *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Enterobacter aerogenes* from Chesapeake Bay in Maryland associated with antibiotic resistance which caused failure in treatment. *Chromobacterium violaceum* isolated in this study is a unique facultative flagellated Gram-negative bacterium, widespread in soil and water (Darmawan *et al*., 2018). Outbreak caused by *C. violaceum*, an opportunistic pathogen, was reported which may cause fatal infections to human or animals (Darmawan *et al*., 2018). *Acinetobacter* spp., *Bacillus* spp., *Staphylococcus* spp., and *Citrobacter* spp. are group of bacteria regularly found in the environment.

The study aimed to study the anthropogenic antibiotic resistance among the heterotrophic bacteria isolated from the rivers. Results showed most of the potential pathogen isolated from the rivers are resistance to most clinically tested antibiotics, especially ciprofloxacin, ampicillin and piperacillin (Table 3). Generally, R10 were the only sites where the bacteria were resistance to all the antibiotics tested, R5 were the only sites where the bacteria were only resistance to ampicillin. Ciprofloxacin is a second-generation, broad-spectrum fluoroquinolone used in treating bacterial associated urinary tract infections (Sharma *et al*., 2017). The alterations of quinolone enzymatic targets due to specific mutation of gyrase or topoisomerase IV are the regular exit pathway adducted by antibiotic resistance associated Gram-negative bacteria (Aldred *et al*., 2014). The findings are in accordance with Hamed *et al*. (2018) who described various ciprofloxacin resistance were detected among Gram-negative bacteria in patients suffering from cancer in Egypt by efflux pump inhibitor. Our results agreed with Azzam *et al.* (2017) and Purohit *et al.* (2017). Bacteria isolated in the study are predominantly susceptible to tetracycline. Tetracycline-class antibiotics have been used for the last 60 years to treat serious life-threatening Gram-negative bacteria causing infections. The result observed was opposed with the previous studies which reported tetracycline resistance bacteria in fish farms and seawater (Hedayatianfard *et al*., 2014).

Table 2. The 16S rRNA sequencing homology search (BLAST) results of the heterotrophic bacteria isolated from 10 rivers in Bintulu, Sarawak

Number of isolates (n)	Bacteria species	Percentages (%)	Database	Similarity (%)
13	*Enterobacter ludwigii*	13	GenBank	99
20	*Enterobacter cloacae*	20	GenBank	98
7	*Enterobacter asburiae*	7	GenBank	99
7	*Acinetobacter* sp.	7	GenBank	99
6	*Acinetobacter nosocomialis*	6	GenBank	98
7	*Bacillus amyloliquefaciens*	7	GenBank	99
6	*Bacillus velezensis*	6	GenBank	99
13	*Klebsiella pneumoniae*	13	GenBank	99
7	*Staphylococcus sciuri*	7	GenBank	99
7	*Chromobacterium violaceum*	7	GenBank	99
7	*Citrobacter youngae*	7	GenBank	99

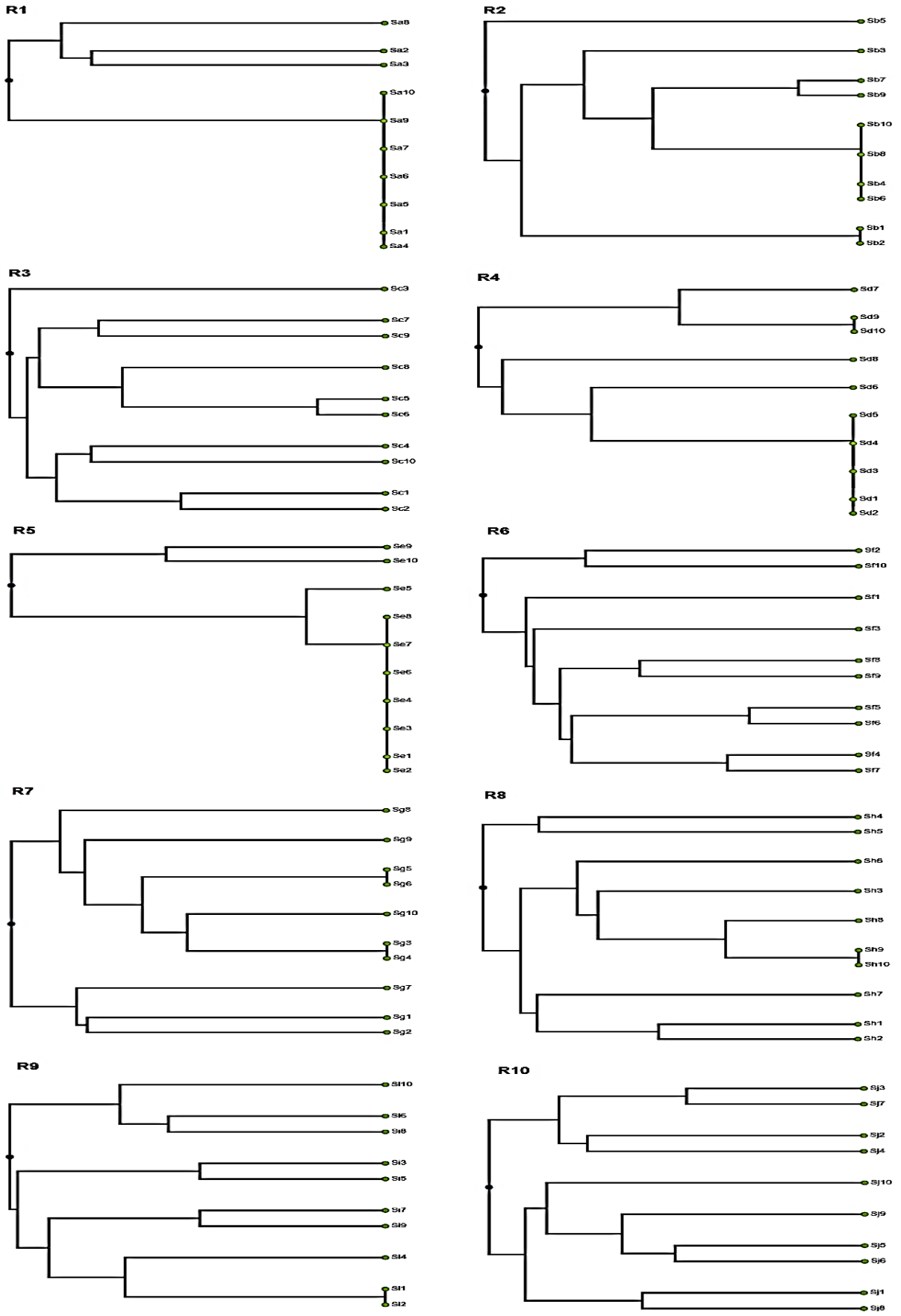

Figure 1. Dendograms of cluster analysis obtained from bacteria DNA gel electrophoresis banding patterns of the (GTG)5-PCR fingerprints, constructed using UPGMA (unweighted pair group method with arithmetic mean) for diversity analysis of heterotrophic bacteria isolated from 10 rivers in Bintulu; Sarawak; R1-R10: sampling sites.

Table 3. Antibiotics susceptibility test among the heterotrophic bacteria isolated from ten rivers in Bintulu, Sarawak against 10 tested antibiotics (n = 100)

Study Site	Resistance rate (%)									
	CIP	C	T	CAZ	SXT	S	PRL	AMP	AK	ATM
R1	100	33	0	0	0	33	66	33	66	66
R2	20	20	0	20	0	0	60	60	0	20
R3	50	0	0	16	0	30	66	83	33	16
R4	80	80	0	80	20	20	80	80	40	100
R5	0	0	0	0	0	0	0	33	0	33
R6	50	0	0	33	13	13	13	66	0	13
R7	100	15	0	75	0	15	86	86	15	86
R8	71	15	0	57	0	29	43	57	29	71
R9	83	17	0	17	0	33	100	100	0	50
R10	83	17	33	17	17	33	67	67	17	29
Mean[1]	63.70 ± 33.40[a]	19.70 ± 23.66[b]	3.30 ± 10.44[c]	31.50 ± 29.20[b]	5.00 ± 8.22[c]	20.60 ± 13.16[c]	58.10 ± 31.37[a]	66.50 ± 21.95[b]	20.00 ± 22.11[b]	48.40 ± 30.95[a]

Legends: R1: Sungai Sibiu, R2: Waterfront, R3: Sungai Plan, R4: Sungai Kemena, R5: Sungai Teknik, R6: Jeti Penambang, R7: Sungai Kirana, R8: Sungai TPU UPM, R9: Sungai Kemunting, S10: Tanjung Batu (SingKwong); [1] The means with the same letter superscript are not significantly different at the 5% level; Antibiotics tested: Ciprofloxacin (CIP), Chloramphenicol (C), Tetracycline (T), Ceftazidime (CAZ), Sulphamethoxazole trimethoprim (SXT), Streptomycin (S), Piperacillin (PRL), Ampicillin (AMP), Amikacin (AK), Aztreonam (ATM).

CONCLUSION

Our findings highlight multiple antibiotics resistance and microbiological analysis (high bacteria diversity and fecal coliform) of heterotrophic bacteria in polluted aquatic ecosystems, thus hygienic condition of most rivers in Bintulu city are getting worst and polluted. Detection of possible pathogens such as *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Chromobacterium violaceum* which acquired multiple antibiotic resistance property are the utmost concern. The present study provides baseline data which help in exploring the mechanisms underlying this inconsistency between antibiotic resistance and pollutant will be a fascinating topic for the upcoming research. The preservation of the river is important as hydrologic reasons, maintain the microbial framework, ecological integrity of the river.

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