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Research Article

Water Quality Analysis and The Occurrence of Antibiotic-Resistant Bacteria (ARB) From Satow Waterfall in Bau, Malaysian Borneo

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ABSTRACT

Water quality monitoring of a particular river is crucially important to determine if the river water meets the requirement for its designated uses. Therefore, an assessment of the physiochemical water quality at Satow Waterfall, Sarawak, and the prevalence of antibiotic-resistant bacteria (ARB) was conducted to corroborate the safety of the water from the waterfall. A total of 42 water samples were collected at the subsurface water of the upstream, midstream, and downstream on two sampling trips in June 2021 (dry season) and December 2021 (wet season), with six months intervals. The water quality index (WQI) of the sampling stations ranged from 92.60 to 95, classifying the water under Class I. However, the water is microbiologically polluted, with the highest coliform count of 2.59×10^5 CFU/mL recorded at the middle stream during the wet season, which has surpassed the regulatory standard set by the Malaysian Department of Environment (DOE). A total of 54 bacterial isolates were chosen as representatives and identified by 16S rRNA sequencing, which confirmed the existence of 21 bacterial genera. All identified isolates were tested against 15 antibiotics of various classes employing the Clinical and Laboratory Standards Institute (CLSI) protocols. The antibiotic susceptibility test (AST) demonstrated substantial resistance to sulfamethoxazole (48%) and erythromycin (48%), as well as high susceptibility to chloramphenicol (84%), levofloxacin (60%) and ofloxacin (60%). The Multiple Antibiotic Resistance Index (MARI) evaluations demonstrated the MARI varied from 0-0.60, with 33% of the isolates having a MARI greater than 0.2. In this study, the occurrence of ARB in a water environment where there is an undisclosed source of antibiotic utilization is still inevitable, highlighting the necessity to constantly monitor the water quality and the pervasiveness of ARB in recreational water.

Key words: 16S rRNA sequencing, antibiotic resistance bacteria, Multiple Antibiotic Resistance Index, recreational water, water quality

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INTRODUCTION

Recreational water illnesses (RWI) are diseases that people can contract from swimming and playing in polluted water, such as waterfalls, rivers, lakes, pools and waterpark. Diarrhea, rashes on the skin, earache, coughing or congestion, and eye pain are the most prevalent symptoms. The disease can be transmitted through germ-infested water contact, such as inhaling its mist (Centers for Disease Control and Prevention, 2021). Though many domestic and wild animals are documented to be diseases reservoirs, water-based recreational activities are gradually taking the limelight in disease transmission (Garba *et al*., 2018).

Exposure to faecal water contamination is linked to acute gastrointestinal sickness,-such as self-recovering gastroenteritis, according to available reports (Centers for Disease Control and Prevention, 2021). Since bacteria are prevalent in muddy areas and shallow water, it has been shown that young children are more likely to get sick from recreational water since they may ingest more water while swimming (Wade *et al*., 2022). Certain RWI can result in mortality, as happened in Lubuk Yu when catastrophic leptospirosis infections endangered people's lives. (Sapian *et al*., 2012). The risk of contracting leptospirosis is significant, as shown by a study by Ghazali (2019) in which environmental samples were taken from a variety of water recreation areas in Johor, Malaysia. The frequency of rats, as indicated by a rat density index (abundance index of rats) between 3.3% - 12.2%, may have increased the risk of leptospirosis transmission in the studied area. Although no Leptospira was detected in the rat samples, it is possible that the germs are prevalent in other rodents. In light of these findings, it is clear that monitoring the microbial content of rivers used for recreation is crucial.

Bacterial infections are typically treated with antibiotics in both human and veterinary medicine. The disposal of antibiotics to the environment is largely caused by human activity even after they have been administered. Optimizing and regulating the use of antibiotics is essential to reducing environmental pollution since antibiotics used in human and animal therapy are dispersed in urine and excreta (Polianciuc *et al*., 2020). Furthermore, numerous researchers have reported finding antibiotics in a wide variety of environmental settings, including municipal sewage, sewage treatment facilities, surface and river water, drinking water, sludge, manure, agricultural soil and sediments (Kraemer *et al*., 2019; Teo *et al*., 2022). Antibiotic-resistant bacteria and ARGs (antibiotic resistance genes) may be able to remain and proliferate in ambient recreational waters, making these bodies of water a potential recipient as well as a natural reservoir for these pathogens. Contact with ARB and ARGs may put bathers in danger, reducing their ability to combat infections.

The downside of Sarawak's water quality monitoring programme is that the authorities only surveil a few rivers (Mohammad Hamdi *et al*., 2022). This means that the government does not keep track of small rivers or other water-based recreational spots, which are often well-known for providing water-based leisure options. To the best of our knowledge, there have only been a select few studies that have conducted the water quality and ARB monitoring for rivers that are used for recreational purposes. Therefore, an investigation was carried out at Satow Waterfall, which is situated in Kampung Singai, Bau. Monitoring offers the verifiable assessment that is required to make appropriate decisions regarding the management of water quality both now and in the future. It also preserves the beneficial uses of the water as well as to identify new and ongoing problems as well as ensuring adherence with the Malaysia Department of Environment (DOE) regulations. This was done in light of the possibility that water, which is typically used for recreational purposes, could act as a medium for the transmission of disease.

The aim of this study is to evaluate the water quality of the Satow Waterfall via physiochemical and biological evaluations employing the Water quality index (WQI) suggested by the DOE as well as to characterize bacterial isolates based on the degree to which they are sensitive to various antimicrobial drugs. The outcomes of this research will be utilized to begin a monitoring program of water quality including the predominance of bacteria that are resistant to antibiotics in Kuching's recreational waters. This will contribute to ensuring that the state's recreational spots continue to be free of harmful contaminants and are safe for use by the general public. As a result, a dataset with water quality assessments and antibiotic resistance trends can be developed, which will be valuable and instructive for future managerial decisions.

MATERIALS AND METHODS

Sampling site

The Satow Waterfall is situated in Kampung Bobak, Singai, Bau. The global positioning system (GPS) coordinates of the sampling stations were upstream (1.54166, 110.18638), middle stream (1.54125, 110.18664) and downstream (1.54097, 110.18672). All stations are mainly utilized for recreational purposes. The location of the sampling site on the map is shown in Figure 1.

Water sample collections

The sample collection took place during the dry (June 2021) and wet (December 2021) seasons, when the weather was sunny in the mornings wand hen most recreational activities take place (9.00 am to 11.30 am). In accordance with Huys (2013) recommendations, the water samples were collected at the subsurface of water - a depth of at least 50 cm below the surface. The collected samples were delivered in a cool box and immediately processed at the Bacteriology Laboratory, Universiti Malaysia Sarawak. Following the preparation of the samples, 100 µL of water samples were filtered using the membrane filtration method on triplicates of Hichrome Coliform agar (Oxoid, USA) and incubated overnight at 37 °C according to the protocol of The American Public Health Association (APHA) 922J 2017.

Water quality assessment

Water quality indicators such as pH, dissolved oxygen (DO) and temperature were monitored in-situ employing the YSI Pro Plus Multiparameter Water Quality. Ex-situ measurements were performed in the Bacteriology Laboratory at Universiti Malaysia Sarawak to determine the levels of ammoniacal nitrogen (NH3-N), chemical oxygen demand (COD), dissolved oxygen (DO), total suspended solids (TSS) and biological oxygen demand (BOD5). The procedures were carried out using APHA (2017) standards. The WQI is calculated using Equation 1, as established by the DOE.

Equation 1:

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

Isolation of bacteria

The procedure outlined by Huys (2003) was utilized to homogenize and process the water samples collected from each sampling station. Following the preparation of the samples, 100 µL of water samples were filtered using the membrane filtration method on triplicates Hichrome Coliform agar (Oxoid, USA) and incubated overnight at 37 °C . The total coliform count (TCC) was calculated Equation 2 (Kumar, 2011) below:

Equation 2:

$$CFU/mL = (\text{number of colonies x dilution factor})/(\text{volume of culture plate})$$

Fifty-four bacterial colonies grown on the agar were inoculated, purified and stored in glycerol for further use at –20 °C.

Fig. 1. The localities of Satow Waterfall in Sarawak, Borneo maps

Bacterial Identification and Characterization

Fifty-four bacterial isolates were identified using 16S rRNA gene-sequencing with primers 27F (5’-CAGGCCTAACACATGCAAGTC- 3’) and 519R (5’-GWATTACCGCGGCKGCTG- 3’) as described by Kathleen et al. (2016). Following the acquisition of DNA sequences generated by Apical Scientific Sdn Bhd, the comparisons of the obtained 16S rRNA sequences with the available nucleotide sequences in the GenBank database were done through BLAST analysis.

Antimicrobial Susceptibility Test (AST)

Antibiotic susceptibility test (AST) was performed according to the established standard from the Clinical and Laboratory Standards Institute (CLSI) on 54 identified bacterial isolates, each of which was tested against 15 antibiotics that belonged to different classes of antibiotics. The bacterial isolates were grown in Mueller Hinton Agar (Oxoid, UK) and the inoculation of bacteria was accomplished by swabbing a sterile cotton swab over Mueller Hinton Agar. Subsequently, the antimicrobial disc was placed on the agar plate and incubated overnight at 30 °C The antibiotic discs (Oxoid, UK) used in this study were streptomycin (S, 10 µg), kanamycin (K, 30 µg), cefotaxime (CTX, 30 µg), cephalothin (KF, 30 µg), tetracycline (TE, 30 µg), doxycycline (DO, 30 µg), carbenicillin (CAR, 100 µg), ofloxacin (OFX, 5 µg), levofloxacin (LEV, 5 µg), sulfamethoxazole (SXT, 25 µg), meropenem (MEM, 10 µg), ertapenem (ERT, 10 µg), vancomycin (VA, 30 µg), chloramphenicol (C, 30 µg) and erythromycin (E, 15 µg). The positive controls used in this study were Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29213.

MAR Index Evaluation

The multiple antibiotic resistance (MAR) index analysis was carried out according to Equation 3 (Krumperman, 1983).

Equation 3:

MAR Index= Total number of isolates showing resistance against antibiotics/Total number of antibiotics being tested

Statistical analysis

The physiochemical water quality data are expressed as the mean value of triplicate trials with standard error at the 5% significant level. All experiments were repeated three times except for TSS. The Tukey test was used to conduct the analysis of variance (ANOVA), and mean differences were deemed significantly different if the p-value was 0.05. The IBM SPSS (Version 26) was used for all statistical analyses.

RESULTS

Total coliform count (TCC)

Coliforms are a group of bacteria that are not hazardous to humans. Ingestion of pathogens, which include a wide range of bacteria, parasites, and viruses, can result in health issues. The prevalence of pathogens is determined indirectly by screening for “indicator” microorganisms such as coliform bacteria. Since coliforms are relatively recognizable, they are more prevalent than other dangerous pathogens, and they are sensitive to external factors like wastewater treatment and water purification in a way that many other pathogens do (USEPA, 2022). Table 1 represents the TCC count for each station during dry and wet seasons.

The highest coliform count was determined at the middle stream, 2.59 × 10^5 CFU/mL during the second trip and the lowest TCC count was recorded at the upper stream during Trip 1 with a value of 1.80 × 10^5 CFU/

mL. The high TCC matched the findings of Leong *et al*. (2018), Morales-Durán *et al*. (2018) and Mohammad Hamdi *et al*. (2022). The reason for the highest numbers during the rainy season may be related to the invasion of microorganisms into the dam caused by runoff from sources such as decaying vegetation, rubbish, domestic waste, and faeces (Chouhan, 2015). According to their findings, the highest counts were recorded during the summer months suggesting that the high temperature of the dam's water may have stimulated the growth and reproduction of the organisms, whereas the lowest counts were recorded during the winter months suggesting that the lower temperature of the water may have slowed the growth rate. Thus, seasonality is thought to have been influenced by changes in precipitation and water temperature. A number of other authors have shown very similar results (Agarwal & Rajwan, 2010). Given there is no major land uses adjacent to the sampling site, it is anticipated that the majority of the TCC originates from the recreationists, storm runoff and stray animal faeces. Regardless of the fact that no apparent outbreak has been documented, the disconcerting prevalence of coliform bacteria in the water may constitute a health risk to visitors and vigilant measures must be taken when in contact with water to avert any RWI occurrence.

Physiochemical water quality analysis

The Water Quality Index, an indexing system, was introduced to assist in the sorting of the enormous amount of collected data in compliance with the Interim National Water Quality Standards. As affirmed by Bordalo *et al*. (2006), those indices were developed to make water quality evaluations more accessible. Generally, all stations were classified as Class I water with WQI value ranging from 93-95. Class I water is safe for recreational purposes and there is no conventional treatment needed. Other than that, the middle stream's water was classified as Class II water during the second trip. Table 2 shows the results of the physiochemical analysis of the water of Satow Waterfall.

All water quality parameter values are within the acceptable range, with the exception of BOD_5, and are in accordance with those specified by DOE, Malaysia. Recreational activities, such as picnicking, upstream, midstream, and downstream of the Satow Waterfall, have been associated to an elevation in BOD_5. The concentration of BOD_5 may rise as a result of the emission of phosphate and nitrate. Dishwashers, drinks, and food additives could all be contributing to phosphate leaks. This assumption is supported by Mohammad Hamdi *et al*. (2022), who also asserted that recreational activities may lead to chemical release, which can decrease the BOD_5 level in the water environment. Water quality index fluctuations in rivers have been significantly proven to be correlated with human activity. Lee-Goi (2020) performed a comprehensive study contrasting water quality indicators in Malaysian rivers before and after the adoption of the Movement Control Order (MCO) to provide evidence for the assertion. The quality of the river's water improved during MCO due to restrictions on commercial activity, human mobility and anthropogenic activities. It was concluded that over the course of the MCO periods, Malaysia's river water quality index has improved, showing the relationship between the involvement of human activities and the water quality status of river. An environmental impact assessment (EIA) must be conducted in the future in order to assess the potential pollutants that might be related to the physiochemical and microbiological status of Satow Waterfall.

Identification of bacteria

From the 16S rR NA sequencing, this study revealed the prevalence of 21 distinct bacterial genera throughout both seasons: Enterobacter (14%), Klebsiella (13%), Bacillus (9%), Staphylococcus (7.4%), Citrobacter (7.4%), Alcaligenes (5.5%), Shigella (5.5%), Acinetobacter (3.7%), Brevibacillus (3.7%), Chromobacter (3.7%), Salmonella (3.7%), Escherichia (3.7%), Pseudomonas (3.7%), Proteus (1.8%), Serratia (1.8%), Pectobacterium (1.8%), Comamonas (1.8%), Lelliota (1.8%), Stenotrophomonas (1.8%), Kosakonia (1.8%) and Bifidobacterium (1.8%). The opportunistic pathogens *P. aeruginosa*, *Enterobacter* spp., *K. pneumonia*, *S. saprophyticus, C. haemolyticum*, *Salmonella* spp., *Shigella* spp., *E. coli, P. mirabilis*, and *Citrobacter* spp. were among some of the detected bacterial species. Table 3 and Table 4 show the identity of each bacterium isolated from Satow Waterfall samples during dry and wet season, respectively.

Recent data and statistics on bacterial distribution in Sarawak's waterfall environment are scarce, to the best of our knowledge. Due to obsolete information, more study is required to determine how bacteria spread in waterfall environment. Future public health risk evaluations can benefit from a more in-depth knowledge of how ARG spread in different settings. Eleven of the 21 bacterial genera have been found to belong to the Enterobacteriaceae family. This bacterial family accounts for 53.3% of the total number of bacteria found in Satow Waterfall. The Enterobacteriaceae family of bacteria includes Enterobacter (14%), Klebsiella (13%), Citrobacter (7.4%), Shigella (5.5%), Salmonella (3.7%), Escherichia (3.7%), Serratia (1.8%), Pectobacterium (1.8%), Lelliota (1.8%), Proteus (1.8%), and Kosakonia (1.8%). Enterobacter sp. (14%) predominated all the bacterial taxa that were recovered from Satow Waterfall. The bacterial genus found in this study is consistent with those reported by Staradumskyte and Paulaukas (2012) and Lihan *et al*. (2017).

Etiological aspects of waterborne microbial infections are a public health issue in developing nations, particularly among those with the poorest economic circumstances and sanitation infrastructure. The most important factor, according to many experts and practical users, is to continually guarantee the safety of drinking water at all points in the water supply chain, from the catchment region to the user (Naidoo & Olaniran, 2013). There are many potential contributors of this ecological pollution, notwithstanding the fact that the water already harbors a diverse range of microorganisms naturally. Numerous epidemiological investigations have identified recognized point sources and non-point causes of water pollution (Fewtrell & Kay, 2015). Animal excrement, sand suspension and storm water discharge are examples of non-point causes of pollution. However, Fewtrell and Kay (2015) noted that in the case of non-point source inputs, it is challenging to determine whether pollution is brought about by humans or animals.

Antibiotic susceptibility testing and MAR Index evaluation

The antibiotic susceptibility test was carried out in accordance with the methodology provided by

CLSI. The CLSI’s procedure was followed in order to conduct the test to determine antibiotic susceptibility. The highest percentage of resistance recorded was against erythromycin (41%), sulfamethoxazole (40%) followed by carbenicillin (39%), cephalothin (24%) and doxycycline (22%). Accordingly, the highest susceptibility recorded was against chloramphenicol (92%) followed by levofloxacin (77%) and ofloxacin (77%). Figure 2 is the representative image of halozone formations by 2S42 and S11 isolate.

The antibiotic susceptibility profiles of each bacterium isolated during dry and wet season can be found in Appendix 1 and Appendix 2, respectively. The heterogeneity across bacterial species in terms of antibiotic resistance patterns indicates the presence of selective pressure in aqueous habitats, which is in line with research by Hiltunen *et al*. (2017). Table 5 shows the percentage of the AST response (Resistant, Intermediate and Susceptible) while Table 6 shows the antibiotic resistance pattern of all bacterial isolates discovered in this study.

In some places (including Taiwan, Nigeria, and a few African towns), the rate of SXT-resistance is high, and this contributes to the spread of the strain that carries the dfrA, dfrG, and/or dfrK gene through domestic and farm animals (Sato *et al*., 2021). The high erythromycin resistance observed was consistent with the findings of Ahmadpoor (2021) and Fontana *et al* (2021). The finding of this study is consistent with previous research by Lim and Apun (2013) and Kathleen *et al*. (2016), who also showed that almost none of the tested bacteria were chloramphenicol resistant. The use of chloramphenicol has historically been outlawed in aquaculture in several countries, notably Malaysia, since 1983 since it can cause serious or deadly blood issues even at very low dosages, hence lowering the prevalence of chloramphenicol-resistant bacteria in the environment (Kathleen *et al*., 2016).

Table 1. The TCC for each station

Season	CFU/100mL		
	Upstream	Middle stream	Downstream
Dry	1.80×10^{5} ±9.07	2.47×10^{5} ±5.86	2.10×10^{5} ±13.42
Wet	2.04×10^{5} ±4.65	2.59×10^{5} ±5.94	2.50×10^{5} ±7.22

Table 2. The water quality evaluation results of Satow Waterfall during Trip 1 and 2

Sampling stations	Parameters	Dry Season		Wet Season	
		Value	SI	Value	SI
1 (Upstream)	pH	7.12 ± 0.01^{a}	98.98	6.56 ± 0.01^{a}	97.44
	Temperature	23.6 ± 0.06^{a}	-	24.7 ± 0.10^{a}	-
	TSS (mg/L)	5	94	7	93.34
	DO (mg/L)	7.90 ± 0.01^{a}	100	8.41 ± 0.01^{b}	100
	BOD (mg/L)	2.05 ± 0.01^{a}	91.72	2.65 ± 0.01^{a}	89.19
	COD (mg/L)	3 ± 0.01^{a}	95.11	9 ± 0.58^{a}	87.13
	NH_3-N	0.09 ± 0.01^{a}	91.05	0.11 ± 0.01^{a}	88.95
	WQI	95	I	93	I
2 (Middle stream)	pH	7.26 ± 0.00^{b}	98.34	6.69 ± 0.01^{b}	98.37
	Temperature	23.9 ± 0.01^{b}	-	24.7 ± 0.10^{a}	-
	TSS (mg/L)	11	91.06	12	90.50
	DO (mg/L)	7.85 ± 0.01^{b}	100	8.13 ± 0.01^{a}	100
	BOD (mg/L)	2.55 ± 0.01^{b}	89.61	2.48 ± 0.01^{b}	89.90
	COD (mg/L)	7±0.33b	89.79	7±0.33a	89.79
	NH3-N	0.07 ± 0.01^{a}	93.15	0.14 ± 0.01^{a}	85.80
	WQI	93	I	92.60	II
3 (Downstream)	pH	7.88±0.04c	92.64	6.81 ± 0.01^{c}	99.02
	Temperature	23.9±0.00c	-	24.7 ± 0.10^{a}	-
	TSS (mg/L)	8	92.77	5	94
	DO (mg/L)	7.32±0.01c	95.99	8.12 ± 0.01^{a}	100
	BOD (mg/L)	2.16±0.01c	91.26	2.31 ± 0.01^{c}	90.63
	COD (mg/L)	3±0.01a	95.11	5 ± 0.33^{b}	92.45
	NH3-N	0.15±0.00b	84.75	0.14 ± 0.00^{a}	85.80
	WQI	93	I	94	I

Note: The data are expressed as the standard deviation of the average of triplicates. At the 5% level, there is a considerable difference in the values in each column with the different superscripts ($^{a-b-c}$). There is no statistically significant difference between the numbers that have the same superscript, and vice versa.

Table 3. The bacterial identities of isolates recovered from the subsurface of Satow Waterfall during dry season

Isolate	Bacterial Identification	Accession number
S1	*Acinetobacter calcoaceticus*	CP020000.1
S2	*Proteus mirabilis*	AM942759.1
S3	*Alcaligenes* sp.	OK035312.1
S4	*Brevibacillus* sp.	MK834704.1
S5	*Enterobacter ludwigii*	MT177074.1
S6	*Bacillus badius*	MK995595.1
S9	*Enterobacter* sp.	MN960104.1
S10	*Enterobacter hormaechei*	KP313053.1
S11	*Serratia marcescens*	JQ308610.1
S12	*Pectobacterium carotovorum*	LN851554.1
S13	*Brevibacillus agri*	MK629832.1
S14	*Shigella flexneri*	CP055109.1
S17	*Klebsiella oxytoca*	MN512292.1
S19	*Bacillus* sp.	MH883970.1
S22	*Klebsiella variicola*	CP047360.1
S23	*Citrobacter freundii*	MZ047972.1
S24	*Chromobacterium* sp.	MN122133.1
S25	*Lelliottia amnigena*	KY400219.1
S28	*Citrobacter* sp.	MF148474.1
S31	*Comamonas testosteroni*	MF993019.1
S33	*Klebsiella* sp.	AB269870.1
S34	*Salmonella enterica*	KY971289.1
S35	*Escherichia coli*	AY078065.1
S36	*Klebsiella grimontii*	CP056701.1
S38	*Citrobacter youngae*	MN988876.1
S40	*Enterobacter cloacae*	MT465116.1
S43	*Klebsiella pneumonia*	KJ194593.1
S44	*Enterobacter kobei*	MN691835.1
S45	*Enterobacter* sp.	MK092991.1
S46	*Bacillus pumilus*	MN197953.1
S49	*Enterobacter* sp.	CP005991.1
S48	*Klebsiella quasipneumoniae*	CP055213.1
S53	*Stenotrophomonas* sp.	MH153790.1

Table 4. The bacterial identities of isolates recovered from the subsurface of Satow Waterfall during wet season

Isolate	Bacterial Identification	Percentage of Similarity (%)
2S2	*Staphylococcus gallinarum*	MN192140.1
2S4	*Pseudomonas* sp.	MZ836867.1
2S5	*Shigella flexneri*	CP055109.1
2S7	*Kosakonia radicincitans*	MN841788.1
2S8	*Bifidobacterium longum*	MG654783.1
2S11	*Shigella sonnei*	MW600392.1
2S13	*Bacillus* sp. *SC-C3-2 16S ribosomal RNA gene*	DQ319047.1
2S16	*Acinetobacter soli*	CP016896.1
2S19	*Escherichia coli*	AY078065.1
2S20	*Salmonella enterica*	CP096118.1
2S22	*Alcaligenes faecalis*	MN833525.1
2S23	*Staphylococcus* sp. *strain SZ04*	OM049338.1
2S25	*Bacillus cereus*	HF570080.1
2S31	*Alcaligenes aquatilis*	MT572474.1
2S32	*Staphylococcus saprophyticus*	MT071602.1
2S33	*Klebsiella pneumoniae s*	CP067608.1
2S37	*Enterobacter cloacae*	KT260587.1
2S42	*Chromobacterium haemolyticum*	AP019312.1
2S44	*Staphylococcus edaphicus*	OM049281.1
2S45	*Citrobacter freundii*	FJ608234.1
2S47	*Pseudomonas aeruginosa*	CP000438.1

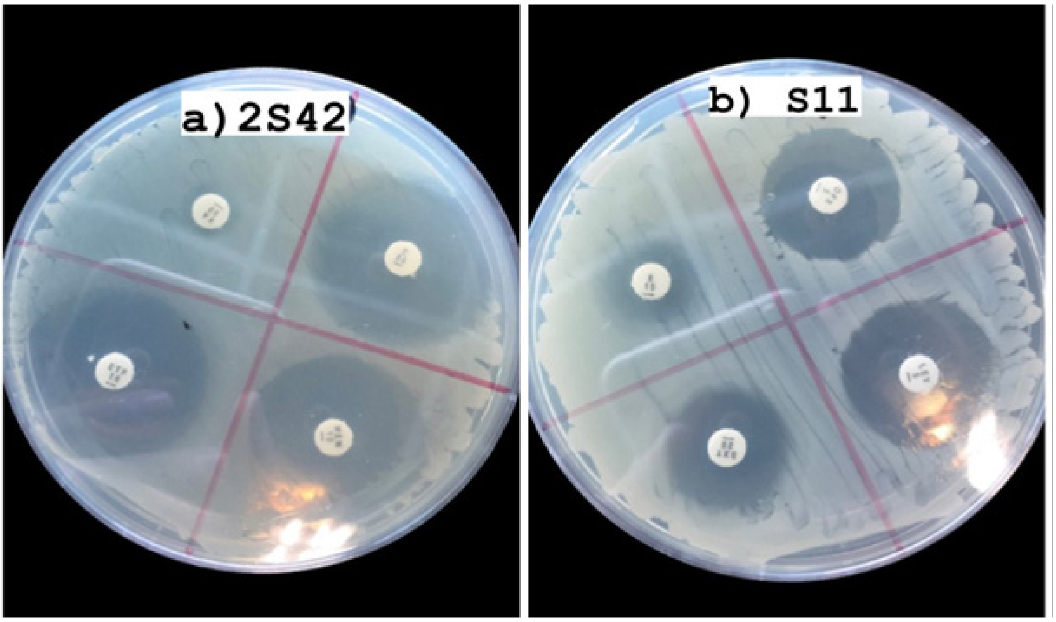

Fig. 2. The representative diagrams of a) The halozone formed by bacterial isolate 2S42 against VA, C, MEM and ETP; b) The halozone formed by bacterial isolate S11 against SXT, E, LEV and OFX.

Table 5. The percentage of resistance according to the types of antibiotics

Antibiotic	Antibiotic's abbreviation	Resistant (%)	Susceptible (%)	Intermediate
Streptomycin	S	19	56	25
Kanamycin	K	19	67	14
Cefotaxime	CTX	20	54	26
Cephalothin	KF	24	46	30
Tetracycline	TE	20	65	24
Doxycycline	DO	22	59	19
Carbenicillin	CAR	39	37	24
Sulfamethoxazole	SXT	40	47	13
Ofloxacin	OFX	13	72	15
Levofloxacin	LEV	7	78	15
Erythromycin	E	41	48	11
Meropenem	MEM	6	61	33
Ertapenem	ETP	13	57	30
Vancomycin	VA	19	67	14
Chloramphenicol	C	7	85	8

Table 6. The antibiotic resistance pattern of bacterial isolates isolated in this study

MAR Index	Antibiotics that the bacteria showing resistance to	Isolates Code	Percentage of Isolates (%)
0.60	S, K, KF, TE, DO, SXT, E, MEM, ETP	S13	2
0.47	S, K, TE, DO, CAR, OFX, LEV	S24	2
0.40	S, K, CAR, SXT, E, VA	S25	2
	S, K, SXT, OFX, LEV, E	S31	2
	CTX, KF, TE, DO, OFX, E	S53	2
	CTX, KF, SXT, MEM, ETP, VA	2S37	2
	S, K, TE, DO, SXT, C	S4	2
0.33	TE, DO, CAR, OFX, LEV	S2	2
	CTX, KF, TE, DO, E	S3	2
	CAR, SXT, E, MEM, ETP	S34	2
	S, K, SXT, OFX, E	2S4	2
	S, K, CTX, KF, E	2S23	2
	TE, DO, SXT, E, C	S13	2
	S, K, CTX, MEM, ETP	S6	2
0.27	TE, DO, SXT, E	2S25	2
	S, K, SXT, E	S23	2
	CAR, SXT, E, VA	S28	2
	TE, DO, CAR, E	2S31	2
0.20	CAR, SXT, VA	S1, S38, 2S16	6
	KF, E, ETP	S11	2
	KF, E, VA	S22	2
	KF, CAR, SXT	S35	2
	CTX, KF, CAR	S49, S46	4
	SXT, OFX, LEV	2S7	2
	TE, DO, E	2S11	2
	S, K, VA	2S42	2
	CAR, SXT, E	2S47	2
	CTX, MEM, ETP	S43	2
	DO, VA, C	S33	2
	CAR, E, C	S48	2
0.13	KF, CAR	S9	2
	CAR, E	S10, S17	4
	CAR, SXT	S12	2
	MEM, ETP	S19	2
	SXT, OFX	2S2	2
	TE, DO	2S5	2
	CTX, KF	2S13, 2S19	4
0.06	CAR	S5, S45, 2S22	6
	CTX	S36	2
	SXT	S44, 2S33	4
	E	2S20, 2S45	4
	VA	S40	2

Antibiotic-resistant bacteria and ARGs may persist and reproduce in ambient recreational waters. Recreators may become vulnerable by contact with ARB and ARGs, which may impair their capacity to combat infections (Mohammad Hamdi *et al*., 2022). In this investigation, 17% (*n*=9/54) of the bacteria were found to be resistant to only one of the antibiotics assayed, whereas 0.06% (*n*=3/54) of the isolates exhibited no resistance to any of the antibiotics examined. Given that water is known to be a hub for bacterial gene exchange in the environment, the discovery of antibiotic resistance in aquatic bacteria in the water sample is not surprising (Igere *et al*., 2022). The majority of isolates (67.0%, *n* = 36/54) have MAR values that are lower than 0.2. Kathleen *et al*. (2016) and Lihan *et al*. (2020) observed very similar outcomes, with the vast majority of isolates coming from sources of low antibiotic contamination 63.1% (*n*=94) and 74.0%, (*n*= 37/50), respectively. Those isolates with MAR indices of 0.2 or below originated from a source with low levels of antimicrobial contamination, as described by Krumperman (1983). This implies that the source of the majority of isolates recovered from Satow Waterfall is less likely to be contaminated by antibiotics.

It has been reported in the scientific literature that ARB and ARGs can be introduced into the environment through a number of different point and nonpoint sources, such as sewage overflows, animal waste, and contaminated wastewater effluents. However, this is not the case with Satow Waterfall. Until recently, the sole known usage of the land around the Satow Waterfall was for recreational and tourism purposes. The waterfall is comprised of a total of six layers, and each tier is being used as a recreational area. For that rationale, the emphasis of this section will be limited to the ways in which recreational use and other environmental factors may affect the prevalence of ARB and ARG at Satow Waterfall.

Antibiotic-resistant microorganisms can be found in regions believed to be undisturbed by human activity (Taylor *et al*., 2017). A wide variety of antibiotics are synthesized by bacteria, fungi and actinomycetes that live in soil and water (Pepper *et al*., 2018). In prior studies, natural antibiotics are likely utilized by microbes for self-defense against competing microbes or for signaling with neighboring microbes, both of which were maintained through genetic mechanisms that were influenced by environmental forces (Zhu *et al*., 2014). Sharing of resistance genes across bacteria is made possible via horizontal gene transfer (HGT), which can be facilitated by a variety of mobile genetic element types such as plasmids, integrons, and prophages (Ben *et al*., 2019). The proliferation of ARB and ARGs in aquatic environments is also likely to be aided by multispecies microbial communities known as biofilms, which are significant ARG reservoirs (Abe *et al*., 2020).

In addition to naturally existing bacteria, point and nonpoint sources of feculent contamination are also possible sources of ARB bacteria and ARGs in recreational waterways. However, the impact that ARB and ARG pollution in water poses to human health is still disputed. This study also adds to the growing corpus of evidence that suggests bird and terrestrial wildlife may play a role in the dissemination of ARB and ARGs since bird dungs were spotted at the surrounding area of Satow Waterfall. However, the threats that ARB and ARGs transmitted from animal pose to human health have not been adequately addressed due to the paucity of clinically relevant data. In view of the fact that birds migrate such enormous distances, our findings and that of Dolejska and Papagiannitsis (2018) indicated that further analysis into the contribution of birds and animals to the dissemination of ARB and ARGs is necessary. It has been claimed that animal faeces is a major contributor to water pollution, both in the form of direct discharge and as runoff into streams. This hypothesis is reinforced by a significant finding from Wu *et al*. (2018).'s study, which discovered the presence of the mobile colistin-resistance gene mcr-1 in colistin-resistant *E. coli* bacteria retrieved from river water and egret dung and suggested that this gene may disseminate across the ecosystem. This hypothesis is reinforced by a significant finding from Wu *et al*. (2018).'s study, which discovered the presence of the mobile colistin-resistance gene mcr-1 in colistin-resistant *E. coli* bacteria retrieved from river water and egret faeces and suggested that this gene may disseminate across the ecosystem.

Our analyses also propose that another possible avenue for the spread of ARB and ARGs in Satow Waterfalls is the use of hygienic or cleaning items that exhibit antibacterial labelling while partaking in recreational activities. Concerns about the use of domestic hygiene and cleaning products that are marketed as being antibacterial are also being raised in light of laboratory data associating exposure to these products' ingredients, notably triclosan, with the formation of antimicrobial resistance (Westfall *et al*., 2019). Exploratory research by Aiello *et al*. (2005) indicated that antibacterial product consumption did not significantly increase antimicrobial drug resistance after one year and did not affect bacterial tolerance to triclosan. However, Aiello *et al*. (2015) claimed that it is possible that the criteria for the development of triclosan-resistant species will be met with increased and sustained use of triclosan. The significance of triclosan in promoting the development of antibiotic resistance among bacteria has been demonstrated by recent investigations, which support this assertion. According to Westfall *et al*. (2019), high levels of triclosan have been linked to an increase in bacterial tolerance of antimicrobial medicines that are bactericidal for organisms like MRSA and *E. coli.* Triclosan is a chemical included in many common household products like soap, toothpaste, and hand soap that is used to reduce or prevent bacterial infection. Multiple studies have found that using products with triclosan could contribute to the spread of bacteria that are resistant to antibiotics and other types of biocides (Orhan, 2020). This concern stems from research showing that exposure to triclosan can lead to bacterial target mutations that confer isoniazid resistance and designates for mutants with sensitivity to multiple antimicrobial medicines via the formation of multidrug-resistant efflux pumps, as described in Freundlich *et al*. (2009).

It appears from this study that the bacteria tested were resistant to multiple antibiotics, which may be the result of a combination of genes that renders them immune to the therapeutic concentrations of these drugs. There is evidence that some therapeutically relevant resistance genes originated in ambient bacteria, and these genes, collectively known as the resistome, may be transferred to pathogens.

CONCLUSIONS

In conclusion, Satow Waterfall is classified as Class I Water. A Class I river is one that does not need any additional treatment as a water supply and maintains its natural ecosystem, as outlined by the Malaysian National Water Quality Standards. On the other hand, the faecal coliform and total coliform count was discovered to be higher in both of the sampling locations, despite the fact that the water is considered to be clean according to the WQI. Terrestrial animals and leisure activities are two potential sources of faecal contamination seen at both sites. According to this study, a significant portion (67.0%; *n*=36/54) of the bacteria recovered from the Satow Waterfall have MAR indices below 0.2, indicating that the majority of the isolates came from locations with relatively low antibiotic exposure. Nevertheless, 77% (n=42/54) of the isolates exhibited multiple antibiotic resistance, which is concerning given that no known antibiotics were used in the vicinity of the Satow Waterfall. The prevalence of bacterial resistance to several antibiotic classes indicates that such bacteria might serve as repositories for antibiotic-resistant genes. Therefore, a continuous monitoring of antibiotic resistance pattern should be strengthened in order to have a better knowledge of the significant role played by recreational aquatic environments and farmed species in the dissemination of MAR amongst potential pathogens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abe, K., Nomura, N. & Suzuki, S. 2020. Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. FEMS Microbiology Ecology, 96(5): 1-12. https://doi.org/10.1093/femsec/fiaa031

Addgene. 2022. Protocol - How to Create a Bacterial Glycerol Stock. URL from https://www.addgene.org/protocols/create-glycerol-stock/ (accessed 7.10.22)

Ahmadpoor, N., Ahmadrajabi, R., Esfahani, S., Hojabri, Z., Moshafi, M.H. & Saffari, F. 2021. High-level resistance to erythromycin and tetracycline and dissemination of resistance determinants among clinical Enterococci in Iran. Medical Principles and Practice, 30(3): 272–276. https://doi.org/10.1159/000516216

Aiello, A.E., Marshall, B., Levy, S.B., Della-Latta, P., Lin, S.X. & Larson, E. 2005. Antibacterial cleaning products and drug resistance. Emerging Infectious Diseases, 11(10): 1565–1570.

APHA. 2017. Standard Methods for the Examination of Water & Wastewater, Centennial Edition (21st Ed.). America Public Health Association.

Arnold, B.F., Wade, T.J., Benjamin-Chung, J., Schiff, K.C., Griffith, J.F., Dufour, A.P., Weisberg, S.B. & Colford, J.M. 2016. Acute gastroenteritis and recreational water: highest burden among young US children. American Journal of Public Health, 106(9): 1690–1697. https://doi.org/10.2105/ajph.2016.303279

Barber, M. 1961. Hospital infection yesterday and today. Journal of Clinical Pathology, 14(1): 2–10. https://doi.org/10.1136/jcp.14.1.2

Ben, Y., Fu, C., Hu, M., Liu, L., Wong, M.H. & Zheng, C. 2019. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. Environmental Research, 169: 483–493. https://doi.org/10.1016/j.envres.2018.11.040

Bordalo, A.A., Teixeira, R. & Wiebe, W.J. 2006. A water quality index applied to an international shared river basin: The case of the Douro River. Environmental Management, 38(6): 910–920. https://doi.org/10.1007/s00267-004-0037-6

Centers for Disease Control and Prevention. 2022. Swimming-related Illnesses | Healthy Swimming Healthy Water CDC. CDC. URL https://www.cdc.gov/healthywater/swimming/swimmers/rwi.html. (accessed 4.3.22)

Chouhan, S. 2014. Enumeration and identification of standard plate count bacteria in raw water supplies. Journal of Environmental Science, Toxicology and Food Technology, 9(2): 67–73.

Dolejska, M. & Papagiannitsis, C.C. 2018. Plasmid-mediated resistance is going wild. Plasmid, 99: 99–111.

Fewtrell, L. & Kay, D. 2015. Recreational water and infection: A review of recent findings. Current Environmental Health Reports, 2(1): 85–94. https://doi.org/10.1007/s40572-014-0036-6

Fontana, C., Patrone, V., Lopez, C.M., Morelli, L. & Rebecchi, A. 2021. Incidence of tetracycline and erythromycin resistance in meat-associated bacteria: impact of different livestock management strategies. Microorganisms, 9(10): 2111. https://doi.org/10.3390/microorganisms9102111

Freundlich, J., Wang, F., Vilchèze, C., Gulten, G., Langley, R., Schiehser, G., Jacobus, D., Jacobs, W. & Sacchettini, J. 2009. Triclosan derivatives: Towards potent inhibitors of drug-sensitive and drug-resistant Mycobacterium tuberculosis. ChemMedChem, 4(2): 241–248. https://doi.org/10.1002/cmdc.200800261

Garba, B., Bahaman, A.R., Bejo, S.K., Zakaria, Z., Mutalib, A.R. & Bande, F. 2018. Major epidemiological factors associated with leptospirosis in Malaysia. Acta Tropica, 178: 242–247. https://doi.org/10.1016/j.actatropica.2017.12.010

Garba, B., Bahaman, A.R., Bejo, S. K., Zakaria, Z., Mutalib, A.R. & Bande, F. 2018. Major epidemiological factors associated with leptospirosis in Malaysia. Acta Tropica, 178: 242–247. https://doi.org/10.1016/j.actatropica.2017.12.010

Ghazali, M.A.N. 2019. Leptospirosis & Typhoid Outbreak in Johor, Malaysia: Johor Malaysia. LAP LAMBERT Academic Publishing.

Hiltunen, T., Virta, M. & Laine, A.L. 2017. Antibiotic resistance in the wild: an eco-evolutionary perspective. Philosophical Transactions of the Royal Society B: Biological Sciences, 372(1712): 20160039. https://doi.org/10.1098/rstb.2016.0039

Hu, Y., Yang, X., Li, J., Lv, N., Liu, F., Wu, J., Lin, I.Y.C., Wu, N., Weimer, B.C., Gao, G.F., Liu, Y. & Zhu, B. 2016. The bacterial mobile resistome transfer network connecting the animal and human microbiomes. Applied and Environmental Microbiology, 82(22): 6672–6681. https://doi.org/10.1128/aem.01802-16

Igere, B.E., Onohuean, H. & Nwodo, U.U. 2022. Water bodies are potential hub for spatio-allotment of cell-free nucleic acid and pandemic: a pentadecadal (1969–2021) critical review on particulate cell-free DNA reservoirs in water nexus. Bulletin of the National Research Centre, 46(1): 1-15. https://doi.org/10.1186/s42269-022-00750-y

Kathleen, M.M., Samuel, L., Felecia, C., Reagan, E.L., Kasing, A., Lesley, M. & Toh, S.C. 2016. Antibiotic resistance of diverse bacteria from aquaculture in Borneo. International Journal of Microbiology, 2016: 1–9.

Kraemer, S.A., Ramachandran, A. & Perron, G.G. 2019. Antibiotic pollution in the environment: From microbial ecology to public policy. Microorganisms, 7(6): 180. https://doi.org/10.3390/microorganisms7060180

Krumperman, P.H. 1983. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Applied and Environmental Microbiology, 46(1): 165–170. https://doi.org/10.1128/aem.46.1.165-170.1983

Kumar, A. 2011. CFU: Colony forming unit & calculation. Bio-Resource. URL http://technologyinscience.blogspot.com/2011/11/cfu-colony-forming-unit-calculation.html#.YygRkXZBy01 (accessed 5.10.22)

Lee Goi, C. 2020. The river water quality before and during the Movement Control Order (MCO) in Malaysia. Case Studies in Chemical and Environmental Engineering, 2: 100027. https://doi.org/10.1016/j.cscee.2020.100027

Leong, S., Ismail, J., Denil, N., Sarbini, S., Wasli, W. & Debbie, A. 2018. Microbiological and physicochemical water quality assessments of river water in an industrial region of the Northwest Coast of Borneo. Water, 10(11): 1648.

Lihan, S., Jamil, N.A., Jamian, M.A.H., Chiew, T.S., Ajibola, O.O., Justin, S., Benet, F. & Kion, L.N. 2020. Distribution and prevalence of antibiotic resistant bacteria in fish farms in East Malaysia. Malaysian Journal of Microbiology, 16(4): 263-274. https://doi.org/10.21161/mjm.190522

Lim, M.H. & Apun, K. 2013. Antimicrobial susceptibilities of Vibrio parahaemolyticus isolates from tiger shrimps (Penaeus monodon) aquaculture in Kuching, Sarawak. Research Journal of Microbiology, 8(1): 55–62.

Ling, T.Y., Soo, C. L., Phan, T.P., Lee, N., Sim, S.F. & Grinang, J. 2017. Assessment of the water quality of Batang Rajang at Pelagus Area, Sarawak, Malaysia. Sains Malaysiana, 46(3): 401–411.

Mohammad Hamdi, K., Lihan, S., Hamdan, N. & Tay, M.G. 2022. Water quality assessment and the prevalence of antibiotic- resistant bacteria from a recreational river in Kuching, Sarawak, malaysia. Journal of Sustainability Science and Management, 17(5): 37–59. https://doi.org/10.46754/jssm.2022.05.004

Mohd Ali, M.R., Mohamad Safiee, A.W., Thangarajah, P., Fauzi, M.H., Muhd Besari, A., Ismail, N. & Yean Yean, C. 2017. Molecular detection of leptospirosis and melioidosis co-infection: A case report. Journal of Infection and Public Health, 10(6): 894–896. https://doi.org/10.1016/j.jiph.2017.02.009

Morales-Durán, N., de la Torre-González, A., García-Sánchez, V. & Chávez, C. 2018. Estudio de la calidad bacteriológica y parámetros fisicoquímicos del agua del Distrito de Riego 023. Tecnología Y Ciencias Del Agua, 09(1): 53–67. https://doi.org/10.24850/j-tyca-2018-01-04

Naidoo, S. & Olaniran, A. 2013. Treated wastewater effluent as a source of microbial pollution of surface water resources. International Journal of Environmental Research and Public Health, 11(1): 249–270. https://doi.org/10.3390/ijerph110100249

Orhan, M. 2020. Triclosan applications for biocidal functionalization of polyester and cotton surfaces. Journal of Engineered Fibers and Fabrics, 15: 1-11. https://doi.org/10.1177/1558925020940104

Pepper, I.L. & Gerba, C.P. 2015. Cultural methods. Environmental Microbiology, 3: 195–212.

Pepper, I.L., Brooks, J.P. & Gerba, C. P. 2018. Antibiotic resistant bacteria in municipal wastes: is there reason for concern? Environmental Science & Technology, 52(7): 3949–3959. https://doi.org/10.1021/acs.est.7b04360

Polianciuc, S.I., Gurzău, A.E., Kiss, B., Ștefan, M.G. & Loghin, F. 2020. Antibiotics in the environment causes and consequences. Medicine and Pharmacy Reports, 93(3): 231-240. https://doi.org/10.15386/mpr-1742

Sapian, M., Khair, M.T., How, S.H., Rajalingam, R., Sahhir, K., Norazah, A., Khebir, V. & Jamalludin, A.R. 2012. Outbreak of melioidosis and leptospirosis co-infection following a rescue operation. The Medical Journal of Malaysia, 67(3): 293–297.

Staradumskyte, D. & Paulaukas, A. 2012. Indicators of microbial drinking and recreational water quality. Biologija, 58(1): 7-13.

Sato, T., Ito, R., Kawamura, M. & Fujimura, S. 2022. The risk of emerging resistance to trimethoprim/sulfamethoxazole in Staphylococcus aureus. Infection and Drug Resistance, 15: 4779–4784. https://doi.org/10.2147/idr.s375588

Taylor, E., Ferreira, G., Freitas, G., Ferreira, R., de Assis Santos, D. & de Resende-Stoianoff, M. 2017. Screening of antifungal susceptibility in cave-dwelling aspergilli and report of an amphotericin B-resistant Aspergillus flavus. International Journal of Speleology, 46(3): 369–378.

Teo, C.Y., Jong, J.S.J. & Chan, Y.Q. 2022. Carbon-based materials as effective adsorbents for the removal of pharmaceutical compounds from aqueous solution. Adsorption Science & Technology, 2022: 3079663. https://doi.org/10.1155/2022/3079663

USEPA. 2022. Fecal bacteria | monitoring & assessment. EPA. URL https://archive.epa.gov/water/archive/web/html/vms511.html (accessed 6.10.22)

Wade, T.J., Arnold, B.F., Schiff, K., Colford, J.M., Weisberg, S.B., Griffith, J.F. & Dufour, A.P. 2022. Health risks to children from exposure to fecally-contaminated recreational water. PLoS ONE, 17(4): e0266749. https://doi.org/10.1371/journal.pone.0266749

Wu, J., Huang, Y., Rao, D., Zhang, Y. & Yang, K. 2018. Evidence for environmental dissemination of antibiotic resistance mediated by wild birds. Frontiers in Microbiology, 9: 745. https://doi.org/10.3389/fmicb.2018.00745

Zhu, H., Sandiford, S.K. & van Wezel, G.P. 2014. Triggers and cues that activate antibiotic production by actinomycetes. Journal of Industrial Microbiology and Biotechnology, 41(2): 371–386. https://doi.org/10.1007/s10295-013-1309-z