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Surface Water Analyses for Antibiotic Susceptibility of Bacteria Isolated from Estuarine Environment, Turtle Sanctuary and High-Density Industrial Zone in Davao City

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ABSTRACT

The researchers sought to determine the antibiotic susceptibility profile of bacteria isolated from surface water of estuarine and sanctuary environments and high-density industrial zone in Davao City. Conducted as a concerted effort to shed light on the burgeoning problem of antibiotic resistant bacteria with the investigation of the spatial distribution of selected heavy metals in surface sediments of surface water. The surface water sources from the three sampling sites (High-Industrial, Nearshore-Estuarine and Turtle Sanctuary) were found to be contaminated with enteric bacteria, carried out by using selective media agar plate and analyzed using the Kirby-Bauer Test (Zone of Inhibition Test). Further, by determining the Antibiotic Resistance Index (ARI) of the bacterial isolates, this study was able to analyze the proportion of bacterial-resistant determinants in a population, wherein, the three sampling sites have been found to be comparably contaminated by pathogenic enteric bacteria ($p>0.05$). The findings of this research serve as baseline information for government agencies in making necessary guidelines to control the transport and disposal of toxic and hazardous wastes in the area. This shall then serve as a yardstick to adopt an effective water treatment management to mitigate the current prevalence of antibiotic resistant infections.

KEYWORDS: Antibiotic susceptibility, estuarine environment, turtle sanctuary, high-density industrial zone

INTRODUCTION

Rapidly emerging resistant bacteria resulting from use, misuse and outright abuse of antibiotics, endanger the efficacy of antibiotic treatment to treat even the most common treatable infectious diseases. Antibiotic-resistant bacteria are potentially one of the deadliest public health concerns and appear to be rapidly increasing and spreading in the environment. The World Health Organization (WHO) and Center for Disease Control and Prevention (CDC) are predicting severe consequences if there are no proposed initiatives to reduce and control the spread of resistant bacteria (Ventola, 2015).

Surface water serves as major sources of water supply for drinking and domestic purposes which greatly impact public health, as these could serve as reservoirs for transfer of antibiotic-resistant pathogens. Surface water environments are susceptible to promote contamination of residual antibiotics that are released through different

sources, such as leaching agricultural run-offs, sewage discharges and leaching from nearby livelihoods of communities (Manyi-Loh et. al 2018).

Residues of antibiotics reach water bodies through wastes discharged from households, drug manufacturing units, hospitals and poultry industries where antibiotics are used in feeds to improve livestock yield. These antibiotics in water lead to evolution of bacteria that are resistant to antibiotics, which then grow in numbers and spread in the environment. The situation could pose a danger to human health as infection with such kind of resistant bacteria could become untreatable.

As posited by Guoyomard-Rabenirina (2017), approximately 700,000 deaths occur due to antibiotic-resistant bacterial infection each year, both in developed and developing countries. In high-income countries, the continued high rates of antibiotic use in hospitals, the community and agriculture have been suspected as one of the main reasons for the spread of antibiotic-resistant bacteria. On the other hand, in low-income countries, antibiotic resistant bacteria spread easily due to poor hand hygiene between humans in the community and through their environment.

Consequently, accurate identification of bacterial pathogens and determining their drug susceptibility pattern are critical for the efficient management of these antibiotic-resistant strains of infections. The results of Antibiotic Susceptibility Testing (AST) of bacterial isolates can predict the clinical response to treatment and guide selection of antibiotics. Using the Minimum Inhibitory Concentrations (MICs) of bacterial isolates, this determines the lowest concentration of antibiotic that will inhibit its growth. According to the Clinical and Laboratory Standards Institute (CLSI), bacteria are classified as Susceptible (S) wherein bacterial isolates are inhibited when recommended dosage of antibiotics is used; Resistant (R) which implies that bacterial isolates are not inhibited by the recommended dosage of antibiotics and clinical efficacy against the isolate has not been reliable; and Intermediate (I) includes bacterial isolates with MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates (Apley, 2018).

Environmental pollution has obviously occurred in many coastal areas, especially in estuaries and coastal bays with dense human populations in their watersheds. Estuaries are transitional areas between the land and the sea, and between freshwater and saltwater environments, they can be seriously impacted by any number activities (Environmental Protection Agency, US-EPA, n.d.). People have historically viewed estuaries and waterways as places to discard the unwanted by-products of civilization. Pollution due to contaminants that endanger the health and well-being of the surrounding community is probably the most important threat to water quality in estuaries.

Rationale. Antibiotic resistance is a major public health risk that can lead to problems such as increased human illness, suffering and death, increased cost and length of treatments, increased economic and emotional burden on families and healthcare systems. To further compound the problem, bacteria with the highest level of resistance were isolated from the environment, where surface water has been identified as the main receptable reservoir of antibiotics and antibiotic-resistant bacteria (Poonia et al, 2015).

Although wastewater treatment plant significantly reduces the total number of bacteria, numerous studies have demonstrated that treated wastewater may contain antibiotic resistant bacteria, especially Enterobacteriaceae and contribute

to the contamination of surface water. In addition, wastewater is rich in nutrients, antimicrobial substances, and other pollutants such as heavy metals, and thus offer optimal conditions for bacterial development and the spread of antibiotic resistant bacteria by mutation or horizontal gene transfer (Guoyomard-Rabenirina, 2017).

As a concerted effort to shed light on this burgeoning problem of antibiotic resistant bacteria, an initiative to investigate the spatial distribution of selected heavy metals in surface sediments of surface water by Aya-ay, et al. (2017) was conducted to indicate the degree of contamination in the area by evaluating concentrations of various pollutants accumulating in the aquatic resources of the region and stressed that the situation may worsen if no effective measure will be done by concerned authorities.

The Turtle Sanctuary in Davao City engages in the recovery, tagging and rescue of illegally captured and detained marine turtles. It is also declared a protected area for mangroves which are abundant in the area. The survival of the turtle population amid environmental degradation lie on the quality of the water resource in which they inhabit. The growing number of industries and communities surrounding Davao City's catchments in the absence of a centralized waste water treatment plant can lead to deposition of heavy metals both on the surface water and sediments along the typical estuary and sanctuary areas.

Moreover, high-density industrial zone in Davao City pose a significant threat to water quality to nearby communities as there various discharges of untreated waste water from illegal settlements along waterways, poor sewage system and industrial and domestic effluents in the city.

To assess impact of growing economy and communities to the estuarine environment, turtle sanctuary and high-density industrial zone, the researchers of the study sought to conduct an antibiotic susceptibility profile of bacteria isolated from surface water of Davao City, to determine the degree of contamination brought about by discharges of industrial and domestic effluents. In so doing, the findings of this research will generate baseline information for government agencies in making necessary guidelines to control the transport and disposal of toxic and hazardous wastes in the area. Further, this study can be used to strengthen and implement existing public policies in protecting the environment. With emphasis, on the Integrated Tripod Mission, teaching, community service, and research, this study is a crucial mechanism for the researchers to transfer research outcomes to the LGU and other government agencies. There is a need to establish the extent to which the surface water used within the community is contaminated with pathogenic microorganisms. This shall then serve as a yardstick to adopt an effective water treatment management to mitigate the current prevalence of antibiotic resistant infections.

Generally, the researchers of this study sought to determine the antibiotic susceptibility profile of bacteria isolated from surface water of estuarine environment, turtle sanctuary and high density industrial zone in Davao City.

Emergence of antibiotic resistant pathogenic bacteria poses a serious public health challenge worldwide. However, antibiotic resistance genes are not confined to the clinic; instead, they are widely prevalent in different bacterial populations in the environment.

Therefore, to understand development of antibiotic resistance in pathogens, we need to consider important reservoirs of resistance genes, which may include determinants that confer self-resistance in antibiotic producing soil bacteria and

genes encoding intrinsic resistance mechanisms present in all or most non-producer environmental bacteria. While the presence of resistance determinants in soil and environmental bacteria does not pose a threat to human health, their mobilization to new hosts and their expression under different contexts can translate into a problem of huge proportions. Human activities further result in enrichment of such determinants in bacterial populations.

Thus, there is an urgent need to understand distribution of resistance determinants in bacterial populations, elucidate resistance mechanisms, and determine environmental factors that promote their dissemination.

The environment, and especially freshwater, constitutes a reactor where the evolution and the rise of new resistances occur. In water bodies such as wastewater effluents, lakes, and rivers or streams, bacteria from different sources, e.g., urban, industrial, and agricultural waste, probably selected by intensive antibiotic usage, are collected and mixed with environmental species. This may cause two effects on the development of antibiotic resistances: first, the contamination of water by antibiotics or other pollutants lead to the rise of resistances. Second, since environmental species are provided with intrinsic antibiotic resistance mechanisms, the mixture with certain species is likely to cause genetic exchange (Lupo et al 2012).

In this context, the role of phages and integrons for the spread of resistance mechanisms appears significant. Certain species could acquire new resistances from environmental donors and introduce the newly acquired resistance mechanisms into the community. Freshwater appears to play an important role in the emergence and in the spread of antibiotic resistances, highlighting the necessity for strategies of water quality improvement.

Further knowledge is needed to better understand the role of the environment as reservoir of antibiotic resistances and to elucidate the link between environmental pollution by anthropogenic pressures and emergence of antibiotic resistances. Only an integrated vision of these two aspects can provide elements to assess the risk of spread of antibiotic resistances via water bodies and suggest, in this context, solutions for this urgent health issue.

Objectives of the Study

This study sought to determine the antibiotic susceptibility profile of bacteria isolated from surface water of estuarine and sanctuary environments and high-density industrial zone in Davao City. The study was comprised of a range of activities including determination of sampling points, field sampling, laboratory analyses, evaluation and reporting of results to raise awareness on degree of contamination in the various sampling areas of interest. There were three (3) sampling points that will be clustered according to the following criteria and wastewater catchment: High Density Industrial Zone; Typical Estuary environment and Turtle Sanctuary. Surface water samples from the pre-determined stations were collected in previously cleaned and dry bottle using the direct method. Safe handling protocol and transport mechanisms were observed. All isolated and identified group of bacteria from water samples were cultured using selective media agar plate and analyzed using the Kirby-Bauer Test (Zone of Inhibition Test). Consequently, the Antibiotic Resistance Index (ARI) were calculated to determine the extent of contamination in terms of exposure

to antibiotics indicated by prevalence of bacterial-resistant determinants sampled from three sampling sites.

METHOD

Research Design. This study employed an analytic observational research design, where the researchers measured the exposure of the samples under study, that is determine the antibiotic susceptibility profile of bacteria isolated from surface water of estuarine and sanctuary environments and high-density industrial zone in Davao City. Observational studies investigate and record exposures or risk factors, in this case, exposure to antibiotics and observe outcomes, in this case, antibiotic susceptibility as they occur. Moreover, an analytic study attempt to quantify the relationship between the effect of an exposure on an outcome and in this study, it is considered to be observational, wherein the researchers of this study merely gathered some water samples to measure effect of plausible exposure on outcome. Evidently, no intervention was conducted and there was passive involvement of researchers (CEBM, 2020).

Research Locale. The project area covers Davao City. There were three (3) sampling points that were clustered according to the following criteria and wastewater catchment: Estuarine Environment, Turtle Sanctuary and High Density Industrial Zone.

Sampling Procedure. Water sample containers were soaked in dilute solutions of hydrochloric acid (HCl), followed by dilute nitric acid (HNO_3) and finally will be rinsed with deionized water prior to use. Surface water samples from the pre-determined stations were collected in previously cleaned and dry bottle using the "direct method." Water samples were collected about 1-2 cm in depth to avoid surface debris until the container is full, then capped and properly labeled with the sampling station, time and date. Collected test materials were placed in water cooler about 4^0C for preservation (US EPA, October 2009). Samples were immediately transported to Science Resource Center – University of Immaculate Conception for laboratory testing.

Analytic Procedure. The antibiotic susceptibility test was used to determine if the wastewater samples were related to a higher level of bacterial antibiotic resistance Escherichia coli and other pathogenic bacteria in the three sampling sites. The surface water was collected from the three sampling sites in sterile screw capped bottles for antibiotic susceptibility test. All samples were then placed in a portable icebox and brought to the laboratory within two hours of collection.

All isolated and identified group of bacteria from water were cultured using selective media (cetrimide agar) plates. Three colonies were isolated from each sampling site and transferred in to 3mL of sterile distilled water to prepare bacterial suspension. Aliquots of 100 μL from each suspension were spread-plated on Mueller-Hinton agar plates. Antibiotic discs were applied on to the plates using sterile needles and the plates were incubated at 37^0C for 24 hours. After incubation, the antibiotic inhibition zone diameters (IZD) was measured. The results obtained were then used to classify isolates as being resistant, intermediate resistant, or susceptible to a particular antibiotic using standard reference values according to Clinical and Laboratory

Standards Institute (CLSI). Multiple antibiotic resistance (MAR) phenotypes were generated for isolates that showed resistance to 3 or more antibiotics.

To test for antibiotic resistance, the following antibiotic discs at the final concentrations that were indicated were aminoglycosides (gentamycin 10 mcg), ß-lactams (amoxicillin 10 mcg, ampicillin 10 mcg, methicillin 10 mcg and penicillin-G 10 mcg), glycopeptides (vancomycin 10 mcg), macrolides (erythromycin 10 mcg), quinolones (ciprofloxacin 10 mcg), tetracyclines (tetracycline 10 mcg) and others (chloramphenicol 10 mcg).

To determine the antibiotic susceptibility profile of identified pathogenic microorganisms in terms of antibiotic resistance sampled from three sites: i. Estuarine environment; ii. Turtle sanctuary; and iii. High-density industrial zone; the antibiotic inhibition zone diameters (IZD) was measured. The results obtained were then used to classify isolates as being resistant, intermediate resistant, or susceptible to a particular antibiotic using standard reference values according to Clinical and Laboratory Standards Institute (CLSI). Multiple antibiotic resistance (MAR) phenotypes were generated for isolates that showed resistance to 3 or more antibiotics.

Statistical Tools. To determine the extent of contamination in terms of exposure to antibiotics indicated by prevalence of bacterial-resistant determinants sampled from three sampling sites, the antibacterial resistance index (ARI) of each location was then calculated according to Hinton, et al. 1985 (as cited by Azzam & El-Dougdoug, 2017), using the formula ARI=y/nx, where 'y' represents the actual number of resistance determinants that will be recorded from a population of size 'n' and 'x' as the total number of antibacterial that will be tested in the sensitivity test.

To test whether there is a significant difference in the extent of contamination between susceptible and resistant bacteria isolates from the three sampling sites of this study, the One-Way Analysis of Variance (ANOVA) was calculated and results interpreted, thereafter.

Ethical Considerations. This study was conducted with a strong commitment to ethical considerations, including transparent communication of study objectives to the sites, obtaining necessary permissions from relevant authorities, and ensuring anonymity through the use of codes. Maintaining the highest level of objectivity throughout the research process was also a fundamental ethical principle, guaranteeing unbiased data collection, analysis, and interpretation. These ethical practices not only protected the rights and privacy of the participants but also fortified the integrity and credibility of the study's findings, reinforcing the responsible conduct of scientific research.

RESULTS AND DISCUSSION

Susceptibility Profile of Bacteria. The researchers of the study sought to determine the antibiotic susceptibility of isolated bacteria present in surface water of three sampling sites: i.) estuarine environment; ii.) turtle sanctuary; and iii.) high-density industrial zone. In vitro susceptibility testing was performed by disk diffusion (Kirby-Bauer) method. The size of the growth-free zone determined whether the bacterium was considered to determine whether the isolated bacteria from the three sampling sites were susceptible, resistant, or intermediate to a particular antibiotic.

Data on the minimum inhibitory concentration (MIC) of various antibiotics used against the isolated bacteria which is defined as the minimum concentration of an antibiotic that is just barely able to prevent the further growth of the infectious organism in vitro were summarized in Tables 1.a, 1.b and 1.c summarizes the results of susceptibility profile.

In this study, the surface water sources from the three sampling sites (High-Industrial, Nearshore-Estuarine and Turtle Sanctuary) were found to be contaminated with enteric bacteria. All bacterial isolates (17) from the highly industrialized zone were found sensitive to co-amoxyclav, ertapenem, cefotaxime, cefotetan, cefuroxime, imipinem, cotrimoxazole, piperacillin -tazobactam, tobramycin and chloramphenicol. However, Stenotrophemonas maltophila demonstrated resistance to antibiotic amikacin. Moreover, Enterobacter sakazii, Enterobacter cloacae, Klebsiella pneumonia, Pasteurella pneumotrophica/haemolytica, Proteus mirabilis and Vibrio fluvialis were found to be resistant to tetracycline. Also, Enterobacter sakazakii, and Klebsiella pneumoniae depicted resistance to antibiotic amipicillin-sulbactam. A specific resistance to lefoxitin was demonstrated by Enterobacter cloacae and Klebsiella pneumonia. Entrobacter cloacae was found resistant to ampicillin.

The bacterial isolates from the nearshore- estuarine showed sensitivity to all other antibiotics except meropenem, and tetracycline.

Lastly, the bacterial isolates from the turtle sanctuary showed antibiotic resistance towards meropenem ,doripenem, tetracycline, ampicillin-sulbactam, and lefoxitin. Specifically, Burholderia cepaciae and Chrysomonas luteola showed resistance to Meropenem while Serratia marcescens to doripenem. Enterobacter aerogenes, Enterobacter sakazii, and Serratia marcescens were found resistant to tetracycline. Only Enterobacter sakazii was resistant to amipicillin –Sulbactam and Enterobacter cloacae, Enterobacter sakazii and Klebsiella pneumonia were found resistant to Lefoxitin. There were also bacterial isolates that showed intermediate susceptibility to Doripenem like Chrysomonas luteola, Enterobacter cloacae, Enterobacter aerogenes and Stenotrophomonas maltophilia. Enterobacter aerogenes demonstrated intermediate susceptibility to amikacin-sulbactam, lefoxitin, and doripenem. Klebsiella pneumoniae was found to have intermediate susceptibility to amplicillin.

Corresponding tables summarize the profile of antibiotic susceptibility of isolated bacteria from the three sampling sites, wherein the values obtained were interpreted to be susceptible, resistant and intermediate according to interpretive standards established by the Clinical and Laboratory Standards Institute (CLSI).

Table 1. a

Susceptibility Profile of Bacteria Isolated from Estuarine Environment

Bacterial Isolates	ANTIBIOTICS																				
	LEV	AMK	AMC	MEM	DOR	ERT	TET	AMS	CTX	LXN	CTN	CEF	IMI	CFZ	CIP	CEF	SXT	PTZ	TOB	CHL	AMP
	Zones of inhibition , mm / SIR																				
Chryseomonas luteola	31	18	-	22/I	21/I	-	-	-	-	-	16	-	-	-	28/I	24/I	-	26	22	-	-
Enterobacter sakazakii	-	23	21	21/I	23	26	11/R	19	30	20	21	23	26	15	30/I	30	21	25	24	25	14
Klebsiella ornithinolytica	30	21	23	24	23/I	25	25	22	30	28	21	25	29	28	30	30	25	24	25	29	28
Pasteyrella pneumotrophica/ haemolytica	-	21	23	24	21	-	21/ I	22	28	22	20	30	25	28	21	27	-	25	25	-	-
Pasteyrella pneumotrophica/ haemolytica	-	17	23	22/I	20/I	-	22	25	28	23	21	31	25	27	30	25	-	21	23	-	-
Proeteus mIrabilis	30	20	24	23/I	25	28	13/R	25	28	30	23	26	30	29	27/I	26	27	25	26	-	-
Providencia Stuartii		25	20	23	21/I	29	17	15	31	22	18	26	24	19	28/I	30	22	26	21	25	25
Pseudomonas aeruginosa	34	15	-	21	18/R	-	-	-	-	-	15	-	-	-	30	25	22	22	-	-	-
Serratia liquefaciens		23	21	25	24	29	18	21	25	24	20	25	29	17	29	29	29	23	25	29	25
Stenotrophomonas maltophilia	30	21	-	21/I	24/I	25	-	-	-	-	14/I	-	30	-	25/I	25	-	22	22	-	-
Vibrio Fluvialis	-	28	21	21/I	25	30	16	25	27	19	22	25	28	26	30	28	29	21	24	24	6/R

[1] data obtained were interpreted to be susceptible, resistant and intermediate according to interpretive standards established by the Clinical and Laboratory Standards Institute (CLSI).

Table 1.b

Susceptibility Profile of Bacteria Isolated from Surface Water of Turtle Sanctuary

Bacterial Isolates	ANTIBIOTICS																				
	LEV	AMK	AMC	MEM	DOR	ERT	TET	AMS	CTX	LXN	CTN	CEF	IMI	CFZ	CIP	CEF	SXT	PTZ	TOB	CHL	AMP
	Zones of inhibition , mm / SIR																				
Burholderia Cepacia	28	22	-	19/R	25	-	-	-	-	-	15/I	29	-	-	-	-	-	25	23	-	-
Chryseomonas Luteola	-	29	33	18/R	21/I	26	21	20	26	25	21	30	30	29	29	27	27	28	21	22	29
Enterobacter Cloacae	-	27	25	25	21/I	26	21	21	29	10/R	21	21	24	24	26	29	22	22	22	25	21
Enterobacter aerogenes		30	22	23	22/I	31	10/R	14/I	27	15/I	14/I	24	30	21	26	25	25	24	22	27	27
Enterobacter Sakazakii		19	20	25	25	26	10/R	11/R	30	10/R	17	26	27	21	30	30	22	26	23	27	28
Hafnia Alvei	-	32	26	19	21	29	21	19	31	30	21	19	25	19	30	27	25	29	30	23	21
Klebsiella pneumonia	-	28	26	27	23	31	20	17	25	11/R	20	22	29	22	26	28	21	25	21	26	15/I
Pasteurella pneumotrophica/ haemolytica		25	25	20/I	26	31	12/I	22	30	25	21	22	27	26	31	29	27	30	22	26	19
Pasteurella pneumotrophica/ haemolytica	-	22	23	20	23	32	13/I	23	31	24	22	22	24	25	30	28	28	21	23	29	21
Stenotrophomonas maltophilia	23	18	-	22	18/I	-	-	-	-	-	24	-	-	-	26	26	-	22	21	-	-
Serratia stuartii	-	25	20	23	21	29	17	15	31	22	18	26	24	19	28/I	30	22	26	21	25	25
Serratia liquefaciens	-	23	20	25	25	29	15/I	23	27	26	20	22	28	16	31	30	27	23	23	30	30
Serratia marcescens	-	21	25	24	19/R	29	11/R	21	23	22	20	19	25	30	25	27	29	30	24	23	27
Shewanella Putrefaciens	-	28	25	21/I	26	25	19	27	29	30	20	30	30	21	29	26	25	29	21	25	28
Stenotrophemonas Maltophila	31	15/I	-	20	21	-	-	-	-	-	14	-	-	-	29	25	-	21	21	-	-

[2] data obtained were interpreted to be susceptible, resistant and intermediate according to interpretive standards established by the Clinical and Laboratory Standards Institute (CLSI).

Table 1.c

Susceptibility Profile of Bacteria Isolated from High Density Industrial Zone

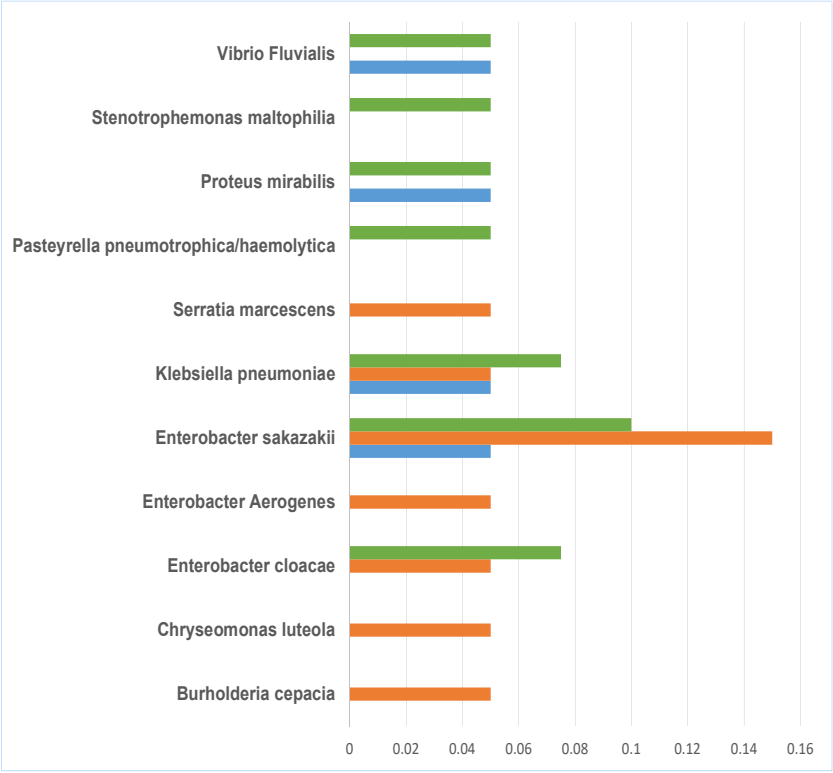
Bacterial Isolates	ANTIBIOTICS																				
	LEV	AMK	AMC	MEM	DOR	ERT	TET	AMS	CTX	LXN	CTN	CEF	IMI	CFZ	CIP	CEF	SXT	PTZ	TOB	CHL	AMP
	Zones of inhibition , mm / SIR																				
Brucella spp		22	27	23	22/I	30	21	27	23	20	20	31	25	26	26	26	22	30	23	22	23
Bukholderia Cepacia	33	30	-	20	21	-	-	-	-	-	15/I	-	-	-	27	22/I	-	30	30	-	-
Enterobacter Sakazakii	-	29	23	23	28	26	10/R	11/R	30	15/I	15.I	22	30	26	29	28	30	23	25	23	30
Enterobacter cloacae	-	21	24	24	29	26	12/R	17	24	19	15/I	22	25	25	28	26	29	23	22	22	12/R
Enterobacter cloacae	-	27	26	23	22/I	25	10/R	22	21	13/R	20	22	25	25	23	29	25	23	23	25	22
Klebsiella pneumonia	-	21	23	23	25	26	11/R	12/R	25	13/R	21	21	30	20	32	27	21	25	39	26	15/I
Pasteyrella pneumotrophica/ haemolytica	-	28	20	22/I	24/I	23	13/R	21	31	26	22	21	27	21	28/I	29	28	21	25	25	26
Proteus mirabilis	30	20	24	23/I	25	28	13/R	25	28	30	23	26	30	29	27/I	26	27	26	27	25	26
Providencia rettgeri	-	23	24	25	27	25	20	20	25	26	-	29	30	28	27/I	26	23	23	21	27	29
Serratia Liquefaciens	-	28	21	25	27	28	21	21	30	27	14/\I	26	28	27	31	27	29	22	25	25	29
Stenotrophemonas maltophilia	31	12/R	-	20	21	-	-	-	-	-	15/I	-	-	-	27	25	-	24	22	-	-
Vibrio fluvialis	33	19	25	23	26	30	12/R	23	28	29	21	25	29	28	28/I	25	25	24	27	-	-

[3] data obtained were interpreted to be susceptible, resistant and intermediate according to interpretive standards established by the Clinical and Laboratory Standards Institute (CLSI).

Second, the researchers sought to determine the extent of contamination in terms of exposure to antibiotics indicated by prevalence of bacterial-resistant determinants sampled from three sampling sites using the Antibiotic Resistance Index (ARI). Chart 1 summarizes the findings.

Chart 1.

Antibiotic Resistance Index (ARI) of Resistant Bacterial isolates from Three Sampling Sites: Estuarine Environment, Turtle Sanctuary and High- Density Industrial Zone



ARI = A/NY; where A is the total number of resistant determinates recorded in the population, N is the number of isolates in the population, and Y is the total number of antibiotics tested (Mohanta et al, 2014).

The antibacterial resistance index (ARI) was used for analyzing the prevalence of bacterial-resistant determinants in a population at a specific location (Chart 1). An ARI value above 0.2 indicates that isolates are exposed to selectivity due to the presence of contaminants such as antibiotics (Mohanta et al, 2014). Since selective pressure can promote dissemination of the resistance determinants, a population with a high ARI score would have more members carrying resistance genes that were likely

to proliferate or transfer resistance genes to other organisms. In this study, the ARI scores for the three sampling sites had highest ARI values for Enterobcater sakazakii, followed by Klebsiella pneumoniae. For the high-density industrial zone and turtle sanctuary ARI were also high for Eneterobacter cloacae. While, Proteus mirabilis and Vibrio fluvialis had also high values for estuarine environment and high-density industrial zone. *Serratia marcescens, Enterobacter aerogenes, Chrysemonas luteola and Burholderia cepacia* were also above ARI 0.02 for turtle sanctuary sampling site.

Both turtle sanctuary and high density industrial zone had higher ARI values than estuarine environment, probably due to that estuaries are semi-enclosed coastal bodies of water that have a free connection with the sea and within which sea water is measurable diluted by fresh water coming from streams, rivers and even groundwater for some areas. The difference in ARI scores for turtle sanctuary and high-density industrial zone may be partially attributed to the latter which may collect a much greater volume of water including storm water that may dilute the antibiotic concentrations from municipal sources thereby lowering the selection pressure.

Lastly, to test the significant difference in the extent of contamination between susceptible and resistant bacteria isolates from the three sampling sites of this study, the One-Way ANOVA was computed.

Table 2.
Statistical Difference of the Antibiotic Resistance Indices (ARI)

ANOVA Summary					
Source	Degrees of Freedom (DF)	Sum of Squares (SS)	Mean Square (MS)	F-Stat	P-Value
Between Groups	2	0.0006	0.0003	0.4377	0.6535
Within Groups	15	0.0109	0.0007		
Total:	17	0.0115			

*Calculation was performed at the 0.05 level of significance

Results of the test showed that the distribution of the identified enteric bacteria according to their Antibiotic Resistant Indices (ARI) from the three sampling sites did not significantly differ ($p > 0.05$). Though, the ARI values obtained: turtle sanctuary, high-density industrial zone and estuarine environment (as seen in Chart 1) have varying ARI range values, statistically this is not so. The three sampling sites have been found to be comparably contaminated by pathogenic enteric bacteria.

The bacterial contamination of surface water by antibiotic resistant pathogens increase the risk to human health. Antibiotic resistance is difficult to remove even if the release of antibiotic resistance determinants in the environment is discontinued due because the resistant pathogens may exhibit multiple resistances. Water regulations and control of waste water, garbage, and any other activities that can cause water pollution should be prohibited (not excluding unmanageably scattered pollutions, e.g. farmlands and animal farms). Thus, few new pollutants are discharged into such areas, and the level of antibiotic resistant pathogens will gradually decrease due to gene loss or environmental self-purification. For aquatic environments that mainly receives

pollutants from scattered sources (such as farmlands and animal farms), leaching and runoff events, which are often seasonal, probably cause the change of antibiotic resistance in different seasons. Further study is indicated to examine these variations.

Conclusion. In this study, the investigation revealed contamination of surface water in three distinct sampling sites, namely the High-Industrial, Nearshore-Estuarine, and Turtle Sanctuary areas, by antibiotic-resistant enteric bacteria. The findings showed that bacterial isolates from the highly industrialized zone were sensitive to several antibiotics but demonstrated resistance to specific ones. Similarly, the nearshore-estuarine isolates were sensitive to most antibiotics, except for meropenem and tetracycline. In contrast, bacterial isolates from the turtle sanctuary exhibited resistance to several antibiotics, including meropenem, doripenem, tetracycline, ampicillin-sulbactam, and lefoxitin. The Antibiotic Resistance Index (ARI) revealed the highest ARI values for Enterobacter sakazakii and Klebsiella pneumoniae across all sites. Notably, the distribution of resistant and susceptible bacterial isolates did not significantly differ between the three sites. Furthermore, the study found that the turtle sanctuary and high-density industrial zone had higher ARI values compared to the estuarine environment, possibly due to the latter's greater dilution effect from freshwater sources. This variance in ARI scores between the turtle sanctuary and high-density industrial zone could be attributed to the latter collecting a larger volume of water, including stormwater, which dilutes antibiotic concentrations and reduces selection pressure. The study employed selective media agar plates and the Kirby-Bauer Test for antibiotic susceptibility testing, while the ARI effectively analyzed the proportion of antibiotic-resistant determinants within the bacterial population.

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