Antibiotic Resistance Profile of Bacteria Isolated from Selected Rivers of Cavite

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ABSTRACT

A study was conducted to determine if selected rivers of Cavite harbor microorganisms with resistance to antibiotics. This is in view of the fact that antibiotics, for several years are regarded as wonder drugs and their indiscriminate use and improper waste disposal may contribute to drug resistance. A total of 190 bacteria randomly isolated from nine rivers traversing the province by Espineli and Ilagan (2018) were tested against 100 ppm concentration of ten antibiotics (ampicillin, erythromycin, streptomycin, tetracycline, kanamycin, ofloxacin, clindamycin, oxytetracycline, levofloxacin, and ceftriaxone). Resistance to oxytetracycline and tetracycline was the most common. One hundred eighty-four of the isolates (96.84%) were resistant to at least three antibiotics. Eight antibiotic resistance patterns were exhibited with hepta-antibiotic resistance as the major pattern. Other isolates exhibited hexa-, octa-, penta-, tetra-, tri-, di- and mono- antibiotic resistance patterns with different antibiotic resistance combinations.

Keywords: antibiotics, antibiotic resistance profile, resistance pattern, selective media, drug resistance, hepta-antibiotic resistance

INTRODUCTION

Antibiotics represent one of the most successful forms of therapy in medicine (Lin et al., 2015). Unfortunately, the use of these antibiotics led to the appearance of organisms that can evade them, the so-called antibiotic resistant bacteria (Nain et al., 2015). Antibiotic resistance is the ability of bacteria to withstand the antimicrobial power of antibiotics (Anderson, 2017). It becomes an issue of public health since resistance can be transferred from bacterium to bacterium and bacterial infections can pass from person to person eventually affecting an entire community (Alliance for the Prudent Use of Antibiotics, 2014). It can also be an environmental concern since approximately 80-90% of the ingested antibiotics are not broken down but pass through the body intact and enter the environment as waste, thus retaining their ability to affect bacteria and promoting antibiotic resistance even after they enter the soil or water as a waste product. Antibiotic resistance remains a continuing problem because this reflects a change in genetic make-up (American Academy of Microbiology, 2008) and the adaptability of bacteria to various pressures in addition to being associated with increased morbidity and mortality (Oves & Hussain, 2016).

Rivers are not exempted from threats of contamination by such microorganisms. The condition of rivers in Cavite is something that should be looked into due to the pronouncement of the Department of Environment and Natural Resources (DENR) that as many as 50 of the 421 rivers in the Philippines are already considered "biologically dead" (Greenpeace, 2010). This is also in view of the increased human population, industrialization, agriculture, and man-made activities around these rivers. Resistance exhibited by bacteria to antibiotics may be due to the presence of these substances in the water environment (Ansari et al., 2014). These antibiotics are released into aquatic environments via the excretion of humans and animals in unaltered forms or metabolites of parent compounds (Kummerer, 2009).

Determination of antibiotic resistance profiles of bacteria isolated from rivers can indicate the rivers' degree of contamination with antibiotics and can assess the rivers' present status thus, this study.

METHODOLOGY

Bacterial Isolates

The bacterial isolates tested for antibiotic resistance are the isolates of Espineli and Ilagan (2018) obtained from the different rivers of Cavite.

These bacteria were maintained in Tryptone Glucose Yeast Extract Agar (TGYA) slants. Duplicate copies were preserved as glycerol stocks.

Antibiotic Resistance Testing

The bacterial isolates were tested for resistance to 100 ppm of ampicillin, erythromycin, streptomycin, tetracycline, kanamycin, ofloxacin, clindamycin, oxytetracycline, levofloxacin and ceftriaxone. Each isolate was grown in master plates and replica plated to antibiotic supplemented Nutrient Agar (NA). Plates were incubated at room temperature for 48 hours. Isolates that grew and survived on the said medium regardless of the size of growth were considered resistant (Agustin, 2006).

Analysis of Data

The percentage of antibiotic resistant isolates was computed by dividing the number of antibiotic resistant isolates by the total number of isolates and multiplied by 100.

Antibiotic resistance patterns were obtained by analyzing the number and kind of antibiotics which the isolates were resistant to (Agustin, 2006).

RESULTS AND DISCUSSION

Antibiotic Resistance of Bacterial Isolates

The bacterial isolates displayed resistance to different antibiotics, namely: ampicillin, erythromycin, streptomycin, kanamycin, tetracycline, oxytetracycline, ofloxacin, levofloxacin, clindamycin, and ceftriaxone (Table 1). These antibiotics represented different antibiotic classifications such as ß-lactams, macrolides, aminoglycosides, tetracyclines, fluoroquinolones, lincosamides, and cephalosporins.

As shown in the table, the most common resistance was observed against tetracyclines: 99.47 percent for oxytetracycline and 94.21 percent for tetracycline. There was a big difference in the percentage of bacteria resistant to aminoglycosides particularly between streptomycin and kanamycin. More isolates (93.16%) were resistant to streptomycin while only 13.16 percent were resistant to kanamycin.

Table 1. Percentage of bacteria resistant to different antibiotics at 100 ppm concentration

ANTIBIOTIC CLASSIFICATION	ANTIBIOTIC	PERCENTAGE OF RESISTANT BACTERIA
ß-Lactams	Ampicillin	91.58
Macrolides	Erythromycin	79.47
Aminoglycosides	Streptomycin	93.16
	Kanamycin	13.16
Tetracyclines	Tetracycline	94.21
	Oxytetracycline	99.47
Fluoroquinolone	Ofloxacin	0.53
	Levofloxacin	2.63
Lincosamide	Clindamycin	81.05
Cephalosporin	Ceftriaxone	85.26

Total number of bacterial isolates: 190

Streptomycin is one of the most commonly used broad spectrum antibiotics and due to its widespread use; bacteria can have a higher chance of developing resistance to the drug (SRS Pharmaceutical Pvt. Ltd., 2010). Resistance to fluoroquinolones was very rare, 2.63 percent for levofloxacin and only 0.53 percent for ofloxacin. This very low percentage of resistance to fluoroquinolones may be due to prescribing guidelines which largely recommend this class as second-line agents for use when narrow-spectrum antibiotics have failed in order to maintain their effectiveness (Redgrave et al., 2014).

Table 2 shows the multiple antibiotic resistance of bacterial isolates. The bacterial isolates were found resistant to at least one out of 10 antibiotics tested at a concentration of 100 ppm. More than half (52.63%) and more than one-eighth (18.95%) of the isolates were resistant to seven and six antibiotics, respectively. On the other hand, less than one-eighth were resistant to one up to five (1.58% to 8.42%) and eight (11.58%) antibiotics. In addition, 184 of the 190 isolates can be considered multidrug-resistant bacteria as they were resistant to three or more antibiotics used (Nain et al., 2015).

Multi-drug resistant bacteria are frequently detected in humans and animals from many countries (Doyle, 2014). Infections caused by multi-drug resistant microbes can be difficult to treat and infections may result in increased costs for treatment due to use of more expensive drugs, more complications, higher mortality, and prolonged hospital stays (Collignon, 2012). Aside from humans and animals, multidrug resistance can also be found in the natural environment including soil and surface waters. Even if these bacteria are not known human pathogens, they may constitute a health risk because of their ability to transfer genetic information to pathogenic microbes (Doyle, 2014) through horizontal transfer (Bollin et al., 2015).

Table 3 shows the percentage of bacteria per genus resistant to antibiotics at 100 ppm concentration. Considering the genera with at least ten isolates, *Serratia* was observed to be the most resistant genus to the antibiotics erythromycin (93.75%), clindamycin (100.00%), and ceftriaxone (100.00%). The *Aeromonas* isolates were most resistant to kanamycin (16.33%). Meanwhile, both *Serratia* and *Aeromonas* were the most resistant genera against the antibiotics ampicillin (100.00%) and tetracycline (100.00%). *Staphylococcus* was the most resistant to both fluoroquinolone antibiotics ofloxacin (6.67%) and levofloxacin (6.67%) and *Pseudomonas* to streptomycin (100.00%). In oxytetracycline, almost all the genera with at least ten isolates were resistant to this antibiotic except for *Pseudomonas* (96.88%).

Bacillus was the least resistant genus against the antibiotics streptomycin (80.95%), tetracycline (80.95%), clindamycin (52.38%), kanamycin

Table 2. Multiple antibiotic resistance of bacterial isolates

NO. OF ANTIBIOTIC	NO. OF RESISTANT ISOLATE	PERCENTAGE
1	3	1.58
2	3	1.58
3	4	2.11
4	6	3.16
5	16	8.42
6	36	18.95
7	100	52.63
8	22	11.58

Total number of antibiotics used: 10

Table 3. Percentage of bacteria per genus resistant to antibiotics at 100 ppm concentration

GENUS NAME	TOTAL NUMBER OF ISOLATE	ANTIBIOTIC/PERCENTAGE OF RESISTANT BACTERIA									
		Amp	Ery	Str	Tet	Ofl	Cli	Kan	Oxy	Cef	Lev
Aeromonas	**49**	**100.00**	**87.76**	**97.96**	**100.00**	**0.00**	**83.67**	**16.33**	**100.00**	**81.63**	**0.00**
Bacillus	**21**	**85.71**	**61.90**	**80.95**	**80.95**	**0.00**	**52.38**	**4.76**	**100.00**	**66.67**	**0.00**
Citrobacter	1	100.00	100.00	100.00	100.00	0.00	0.00	0.00	100.00	100.00	0.00
Corynebacterium	**22**	**81.82**	**59.09**	**86.36**	**90.91**	**0.00**	**77.27**	**9.09**	**100.00**	**81.82**	**0.00**
E.coli	7	100.00	100.00	85.71	100.00	0.00	100.00	0.00	100.00	85.71	0.00
Enterobacter	5	100.00	100.00	100.00	100.00	0.00	100.00	0.00	100.00	100.00	20.00
Klebsiella	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00
Micrococcus	3	33.33	66.67	66.67	33.33	0.00	33.33	33.33	100.00	100.00	0.00
Morganella	2	100.00	100.00	100.00	100.00	0.00	100.00	50.00	100.00	50.00	0.00
Proteus	3	100.00	100.00	100.00	100.00	0.00	100.00	0.00	100.00	100.00	0.00
Providencia	1	100.00	100.00	100.00	100.00	0.00	100.00	0.00	100.00	100.00	0.00
Pseudomonas	**32**	**84.38**	**75.00**	**100.00**	**96.88**	**0.00**	**87.50**	**15.63**	**96.88**	**87.50**	**3.13**
Serratia	**16**	**100.00**	**93.75**	**93.75**	**100.00**	**0.00**	**100.00**	**6.25**	**100.00**	**100.00**	**0.00**
Shigella	2	100.00	100.00	100.00	100.00	0.00	100.00	50.00	100.00	100.00	50.00
Staphylococcus	**15**	**93.33**	**73.33**	**93.33**	**93.33**	**6.67**	**73.33**	**13.33**	**100.00**	**93.33**	**6.67**
Unidentified	1	100.00	100.00	100.00	100.00	0.00	100.00	100.00	100.00	100.00	0.00
Yersinia	9	100.00	88.89	100.00	100.00	0.00	88.89	22.22	100.00	100.00	11.11

Amp - ampicillin
Ery - erythromycin
Str - streptomycin
Tet - tetracycline
Ofl - ofloxacin

Cli - clindamycin
Kan - kanamycin
Oxy - oxytetracycline
Cef - ceftriaxone
Lev - levofloxacin

(4.76%), and ceftriaxone (66.67%) while the genus *Corynebacterium* for ampicillin (81.82%) and erythromycin (59.09%). For oxytetracycline, the least resistant was the genus *Pseudomonas* (96.88%).

The resistance exhibited by the bacteria to antibiotics may be due to the presence of these substances in the water environment (Ansari et al., 2014) which may come from different medical and veterinary and agricultural practices (Toroglu et al., 2005) and bacteria's exposure to them (Gupta et al., 2015).

Bacterial resistance to antibiotics is an increasing problem in today's society and this is acquired by a change in the genetic makeup of a bacterium which can occur by either a genetic mutation or by transfer of antibiotic resistance genes between bacteria in the environment (American Academy of Microbiology, 2008).

Aside from the human health risks posed by the presence of antibiotic resistant bacteria in the environment such as the high possibility of resistance being spread by antibiotic bacteria from the environment to related human pathogenic microorganisms through numerous routes suppressing the effectiveness of antibiotics (Threedeach et al., 2012), and the unwanted presence of antibiotics in the bodies of water due to different medical, veterinary, and agricultural practices (Toroglu et al., 2005), concern for the ecological fate and environmental threat of these drugs in the aquatic milieu is becoming a global phenomenon (Kummerer, 2009). Different kinds of antibiotic resistant bacteria are continuously detected in various environments ranging from aquatic to terrestrial ones (Ayandiran et al., 2014). There is a high possibility of resistance being spread by antibiotic resistant bacteria from the environment to related human pathogenic microorganisms through numerous routes thereby suppressing the effectiveness of antibiotics (Threedeach et al., 2012).

Antibiotic Resistance Patterns of Bacterial Isolates

Bacterial isolates exhibited eight antibiotic resistance patterns (Table 4). One hundred of the 190 isolates showed hepta - antibiotic resistance pattern with four different resistance combinations. Thirty six isolates exhibited hexa-antibiotic resistance pattern with six different resistance combinations, 22 isolates showed octa-antibiotic resistance pattern with two different resistance combinations, and 16 isolates displayed penta - antibiotic resistance pattern with 12 different resistance combinations. Only six and four isolates exhibited tetra-antibiotic resistance pattern with five different resistance combinations and tri-resistance pattern with four different resistance combinations, respectively. Three isolates each displayed di- and mono-resistance patterns with two different combinations each. Results revealed that bacteria harbor resistance to various kinds of antibiotics. Selective pressure that develops when antibiotics are present in the environment for an extended period of time (Couce & Blazquez, 2009) can worsen the problem about the spread of antibiotic resistance in bacteria (Bollin et al., 2015). Thus, antibiotic resistance displayed by the bacterial isolates in this study can be attributed to the possibility that antibiotics were present in the rivers where they were isolated. Exposure to antibiotics or indirectly to wastes containing antibiotics resulted in selective pressures for their evolution to antibiotic resistance via different mechanisms.

Bacteria can be resistant to one or more antibiotics without being pathogenic (Voolaid et al., 2012). However, it has been observed that bacteria in the environment have the potential to act as reservoir of antibiotic resistance genes that may be transferred to pathogenic bacteria (Levy, 2012). They can transmit genes to other bacteria in the same environment through horizontal gene transfer which includes transformation, transduction, and conjugation (Bollin et al., 2015).

Table 4. Antibiotic resistance patterns of bacterial isolates

RESISTANCE PATTERN	ISOLATE IDENTITY (Genus)	NO. OF ISOLATES	%	Amp	Ery	Str	Tet	Ofl	Cli	Kan	Oxy	Cef	Lev
Octa – R	Corynebacterium	1	0.53	+	+	+	+	-	+	+	+	+	-
	Pseudomonas	4	2.11	+	+	+	+	-	+	+	+	+	-
		1	0.53	+	+	+	+	-	+	-	+	+	+
	Aeromonas	5	2.63	+	+	+	+	-	+	+	+	+	-
	Staphylococcus	2	1.05	+	+	+	+	-	+	+	+	+	-
	Serratia	1	0.53	+	+	+	+	-	+	+	+	+	-
	Enterobacter	1	0.53	+	+	+	+	-	+	-	+	+	+
	Shigella	1	0.53	+	+	+	+	-	+	+	+	+	-
		1	0.53	+	+	+	+	-	+	-	+	+	+
	Yersinia	1	0.53	+	+	+	+	-	+	+	+	+	+
		2	1.05	+	+	+	+	-	+	+	+	+	-
	Morganella	1	0.53	+	+	+	+	-	+	+	+	+	-
	Unidentified	1	0.53	+	+	+	+	-	+	+	+	+	-
	Total	**22**	**11.61**										
Hepta – R	Pseudomonas	15	7.89	+	+	+	+	-	+	-	+	+	-
	Serratia	13	6.84	+	+	+	+	-	+	-	+	+	-
	Micrococcus	1	0.53	+	+	+	+	-	+	-	+	+	-
	Aeromonas	27	14.21	+	+	+	+	-	+	-	+	+	-
		1	0.53	-	-	+	+	-	+	+	+	+	-
	Corynebacterium	2	1.05	+	+	+	+	-	-	+	+	+	-
		9	4.74	+	+	+	+	-	+	-	+	+	-
	Staphylococcus	5	2.63	+	+	+	+	-	+	-	+	+	-
		3	1.58	+	-	+	+	+	-	-	+	+	+
	Bacillus	7	3.68	+	+	+	+	-	+	-	+	+	-

ANTIBIOTIC RESISTANCE*

*(+) – resistant; (-) – not-resistant

Amp - ampicillin
Ery - erythromycin
Str - streptomycin
Tet - tetracycline
Ofl - ofloxacin

Cli - clindamycin
Kan - kanamycin
Oxy - oxytetracycline
Cef - ceftriaxone
Lev - levofloxacin

Table 4. Antibiotic resistance … *cont'd*

RESISTANCE PATTERN	ISOLATE IDENTITY (Genus)	NO. OF ISOLATES	%	ANTIBIOTIC RESISTANCE*									
				Amp	Eryth	Strep	Tetra	Oflo	Clinda	Kana	Oxy	Cef	Levo
	Enterobacter	4	2.11	+	+	+	+	-	+	-	+	+	-
	Yersinia	4	2.11	+	+	+	+	-	+	-	+	+	-
	Proteus	3	1.58	+	+	+	+	-	+	-	+	+	-
	Providencia	1	0.53	+	+	+	+	-	+	-	+	+	-
	E.coli	5	2.63	+	+	+	+	-	+	-	+	+	-
	Total	**100**	**52.64**										
Hexa – R	*Pseudomonas*	1	0.53	-	+	+	+	-	+	-	+	+	-
		4	2.11	+	-	+	+	-	+	-	+	+	-
		1	0.53	+	+	+	+	-	+	-	+	-	-
		1	0.53	+	+	+	+	-	-	-	+	+	-
	Citrobacter	1	0.53	+	+	+	+	-	-	-	+	+	-
	Serratia	1	0.53	+	-	+	+	-	+	-	+	+	-
		1	0.53	+	+	-	+	-	+	-	+	+	-
	Staphylococcus	1	0.53	+	-	+	+	-	+	-	+	+	-
		1	0.53	+	+	+	+	-	+	-	+	+	-
	Corynebacterium	2	1.05	+	-	+	+	-	+	-	+	-	-
		4	2.11	+	+	+	+	-	+	-	+	+	-
	Aeromonas	5	2.63	+	+	+	+	-	+	-	+	+	-
		1	0.53	+	-	+	+	-	+	-	+	+	-
		2	1.05	+	+	+	+	-	-	-	+	+	-
	Bacillus	1	0.53	+	+	+	+	-	+	-	+	-	-
		4	2.11	+	+	-	+	-	-	-	+	+	-
	E.coli	1	0.53	+	+	+	+	-	+	-	+	+	-
		1	0.53	+	-	+	+	-	+	-	+	+	-
	Yersinia	1	0.53	+	+	+	+	-	-	-	+	+	-
		1	0.53	+	+	+	+	-	-	-	+	+	-
	Morganella	1	0.53	+	+	+	+	-	+	+	+	-	-
	Total	**36**	**19.01**										
Penta - R	*Bacillus*	1	0.53	+	-	+	+	-	-	+	+	-	-

*(+) – resistant; (-) – not-resistant

Amp - ampicillin
Ery - erythromycin
Str - streptomycin
Tet - tetracycline
Ofl - ofloxacin

Cli - clindamycin
Kan - kanamycin
Oxy - oxytetracycline
Cef - ceftriaxone
Lev - levofloxacin

Table 4. Antibiotic resistance ... *cont'd*

RESISTANCE PATTERN	ISOLATE IDENTITY (Genus)	NO. OF ISOLATES	%	ANTIBIOTIC RESISTANCE*									
				Amp	Eryth	Strep	Tetra	Oflo	Clinda	Kana	Oxy	Cef	Levo
		1	0.53	+	-	+	+	-	-	-	+	+	-
		1	0.53	+	+	+	+	-	-	-	+	-	-
	Staphylococcus	1	0.53	+	+	+	-	-	-	-	+	+	-
	Corynebacterium	1	0.53	-	-	+	+	-	-	+	+	+	-
		1	0.53	+	-	-	+	-	+	-	+	+	-
		1	0.53	+	+	-	+	-	-	-	+	+	-
	Micrococcus	1	0.53	-	+	+	-	-	-	+	+	+	-
	Aeromonas	1	0.53	+	-	-	+	-	+	-	+	+	-
		1	0.53	+	-	+	+	-	+	-	+	-	-
		1	0.53	+	+	+	+	-	-	-	+	+	-
		2	1.05	-	-	+	+	-	+	-	+	-	-
	Pseudomonas	1	0.53	-	+	+	+	-	+	-	+	+	-
		1	0.53	+	-	+	+	-	-	-	+	+	-
	Total	**16**	**8.47**										
Tetra - R	Staphylococcus	1	0.53	+	-	+	+	-	-	-	+	-	-
		1	0.53	-	-	+	+	-	-	-	+	+	-
	Aeromonas	1	0.53	+	-	+	+	-	-	-	+	-	-
	Pseudomonas	1	0.53	-	-	+	+	-	-	+	+	-	-
	Corynebacterium	1	0.53	-	-	+	+	-	-	-	+	+	-
	Bacillus	1	0.53	+	-	-	+	-	+	-	+	-	-
	Total	**6**	**3.18**										

*(+) – resistant; (-) – not-resistant

Amp - ampicillin
Ery - erythromycin
Str - streptomycin
Tet - tetracycline
Ofl - ofloxacin

Cli - clindamycin
Kan - kanamycin
Oxy - oxytetracycline
Cef - ceftriaxone
Lev - levofloxacin

Table 4. Antibiotic resistance … cont'd

RESISTANCE PATTERN	ISOLATE IDENTITY (Genus)	NO. OF ISOLATES	%	ANTIBIOTIC RESISTANCE*									
				Amp	Ery	Str	Tet	Ofl	Cli	Kan	Oxy	Cef	Lev
Tri - R	Bacillus	1	0.53	-	-	+	+	-	-	-	+	-	-
		1	0.53	-	-	+	-	-	+	-	+	-	-
		1	0.53	+	-	-	-	-	+	-	+	-	-
		1	0.53	+	-	-	-	-	-	-	+	+	-
	Total	**4**	**2.12**										
Di – R	Bacillus	1	0.53	-	-	-	-	-	-	-	+	+	-
	Micrococcus	1	0.53	-	-	-	-	-	-	-	+	+	-
	Corynebacterium	1	0.53	-	-	+	-	-	-	-	+	-	-
	Total	**3**	**1.59**										
Mono - R	Corynebacterium	1	0.53	-	-	-	-	-	-	-	+	-	-
	Pseudomonas	1	0.53	-	-	+	-	-	-	-	-	-	-
	Klebsiella	1	0.53	-	-	-	-	-	-	-	+	-	-
	Total	**3**	**1.59**										

*(+) – resistant; (-) – not-resistant

Amp - ampicillin
Ery - erythromycin
Str - streptomycin
Tet - tetracycline
Ofl - ofloxacin

Cli - clindamycin
Kan - kanamycin
Oxy - oxytetracycline
Cef - ceftriaxone
Lev - levofloxacin

From an evolutionary perspective, bacteria use two major genetic strategies to adapt to the antibiotic "attack", (a) mutations in gene(s) often associated with the mechanism of action of the compound, and (b) acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer (Munita & Arias, 2016). In mutational resistance, a subset of bacterial cells derived from susceptible population develop mutations in genes that affect the activity of the drug, resulting in preserved cell survival in the presence of the antimicrobial molecule. Once a resistant mutant emerges, the antibiotic eliminates the susceptible population and the resistant bacteria predominate (Munita & Arias, 2016). On the other hand, horizontal gene transfer includes: (a) conjugation-bacterial cells transfer genetic information through direct cell to cell contact via hollow tubes called pili (Depardieu et al., 2007; Brunsima et al., 2003); (b) transformation - bacterial uptake and integration of foreign DNA into their own genome (Etchuuya et al., 2011); and (c) transduction-genetic material is transferred from bacteria to bacteria via a viral vector (Etchuuya et al., 2011).

CONCLUSIONS AND RECOMMENDATIONS

The rivers of Cavite harbor microorganisms mostly belonging to family Enterobacteriaceae. These organisms are found resistant to 100 ppm concentration of antibiotics and exhibit different resistance patterns. No particular genus is associated to specific antibiotic used in the study. This may be because that particular genus is exposed to one or several antibiotics and horizontal gene transfer has occurred in the environment. This phenomenon warrants further study as health implications could not be ignored.

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