INT. J. BIOL. BIOTECH., 18 (1): 29-38, 2021.

ANTIBIOTIC SUSCEPTIBILITY OF MARINE BACTERIA ISOLATED FROM KARACHI COAST

Moazzam Ali Khan[1], Nageen Tayyab[1], Sarah Arif[1], Khalida Faryal Almani[2] and S. Shahid Shaukat[1]

[1]*Institute of Environmental studies, University of Karachi, Karachi-75270, Pakistan*
[2]*Centre for Environmental Science, University of Sindh, Jamshoro, Pakistan*

ABSTRACT

The present study has been carried out to examine the extent of antibiotic resistance in the microbial flora of the marine environment. The antibiotic resistance of the microbes was tested by 'disc diffusion method'. The susceptibility of marine organisms against Cefazolin, Cefixime, Gatifloxacin, Enoxabid, Urixin, Levofloxacin, Ceftizoxime, Cefpirome, Erythrocin and Cefdinir was investigated. This preliminary research suggests that the terrestrial organisms not only harbor the antibiotic resistance but also have the ability to transfer their resistance to indigenous microbial flora of marine environment.

Key-words: Susceptibility, resistance, terrestrial organisms, marine microflora.

INTRODUCTION

Drug resistance is a term used when microbes have mutated / evolved to resist the effect of the drug to which it was previously susceptible. Cosgrove (2006) conducted an inquiry and suggested that bacteria have the ability to reject the effects of an antibiotic to which they were previously sensitive. This resistant occurs either by genetic mutations or by acquiring antibiotic resistance genes which in turn increase the morbidity rates and mortality rates of infections. The sole driving force leading to antimicrobial resistance is antibiotic use. Resistant organisms are selected by antibiotic use by eradicating that portion of the microbial population which is sensitive to the drug. This results in rapid growth (amplification) of the selected organisms in the microbial living space vacated by the death of the susceptible (Hendley, 1997).

Davies and Davies, (2010) conducted a research that proposed that antimicrobial resistance (AMR) poses worldwide threat of growing concern to living things and its surroundings.One of the main reasons is due to bacteria or superbugs which are resistant to drugs.

Another possibility for increased drug resistance lies in the acquisition of tolerance towards higher concentration of the antibiotic. This is assumed to involve repeated recombination of an antibiotic marker into an existing resistance factor. This will lead to several resistance genes being present on this plasmid which via the enhanced gene dosage effect will lead to greater protein being produced which then will lead to the tolerance of higher concentrations of the antibiotic involved.

Testing for antibiotic susceptibility is an important consideration for the treatment of infections, which is used to develop anti-biograms. Antibiotics are being widely used since 1940's to eliminate bacterial infections without disturbing the other biological activities of living system. However, with each passing year, pathogenic bacteria have developed resistance towards antibiotics, which creates difficulty in the treatment of infections. In fact, many bacterial strains have developed multiple resistances against the commercially available antibiotics. Treatment of the infections can become complicated or even impossible due to the rise in microbial resistance to several drugs. This has led to increase in death rates for some contagious diseases (such as tuberculosis). (www.health.fgov.be/WHI3/periodical/months/wwhv2n5tekst/WWH2306984.htm).

It has also been reported that the bacteria of marine origin have developed resistance against antibiotics. The resistance developed in them is mainly attributed through the organisms of terrestrial environment, which ultimately finds its way in the marine environment (Greig *et al* 2015).

The antibiotic resistance in the marine bacteria finally enters the food chain, which is of serious public health concern. This continuously increasing antibiotic-resistance eventually gives rise to the use of second, third and even fourth generation antibiotics.It is with this aim the present investigation is carried out to achieve the following objectives:

a. To isolate the predominant microbial flora of marine environment.
b. To test theantibiotic susceptibility of selected marine bacteria.

c. To study whether the organisms are sensitive, intermediate or resistant to commercially available drugs.

MATERIALS AND METHODS

The Study Area

Five coastal locations in Clifton beach area were selected for sampling - National Institute of Oceanography, Sizzler restaurant, Mc Donald Restaurant, Kinara Restaurant and Village Restaurant

Sea water samples were collected in sterilized glass bottles from above given sites. Two samples from each site were collected from February till October, 2006.

Methodology

The number of dilutions was made from water samples. These dilutions were run on differential and selective media. Nutrient agar was prepared in sea water, to study the cultural characteristics and for the purification of isolates. Initially, Nutrient, EMB and MacConkey agar were used to examine the morphology of different colonies of pathogens growing on agar in a Petri plates. A swab from samples spread directly onto agar, colonies appeared which differed in their shape, size, colour and texture. EMB was selective for gram-negatives.

Enumerative and presumptive identification of predominant colonies were done by Gram staining,Smears were prepared then stained by Gram's Method. They were used to observe the type and number of bacteria. Further identification of isolates was performed by QTS (Quick Testing Strips) at DESTO Karachi Laboratory.

After conducting Gram staining, Gram positive and Gram negative microbes were found. In biochemical testing Catalase and Coagulase tests were performed for Gram-positive organisms *i.e. Staphylococcus* spp. The catalase test is used for distinguishing between streptococci catalase positive or streptococci catalase negative. Coagulase testing method is performed to identify *Staphylococcus aureus.* These organisms produce a unique enzyme that is coagulase and it can be identified by using either the tube coagulase test (TCT) or the slide coagulase test (SCT).

For Gram-negative microorganisms, Oxidase test is commonly used to identify the microbes that contains specific the enzyme cytochrome oxidase. It is also performed to distinguish between oxidase positive Pseudomadaceae and oxidase negative Enterobacteriaceae. For further differentiation, we perform ONPG Test. Enterobacteriaceae family can be differentiate by ONPG Test late lactose fermenters from lactose nonfermenters in the family Enterobacteriaceae. CIT is performed to determine that an organism can utilize citrate or not. Another test is URE Test which is used to differentiate organisms based on ability to hydrolyze urease. Glucose Test is used to determine if an organism can use glucose. Remaining tests performed are as follows:
Indole test (IND) and Voges–Proskauer test (VP)

Antibiotic Sensitivity Test

Mueller-Hinton agar was used for antibiotic susceptibility testing. Antimicrobial sensitivity test for each identified microbial isolate was tested employing 10 antibiotic discs. These procedures are based on the "disc diffusion methods" proposed by Bauer *et al.*(1966). The antibiotics used in the study were all different generations of cephalosporin; Cefazolin, Cefixime, Gatifloxacin, Enoxabid, Urixin, Erythrocin, Cefdinir, Levofloxacin, Cefpirome, Ceftizoxime. "Antibiotic susceptibility testing was performed by using the disc diffusion method" as per standard method describe in J.A.C.,(David and Brown,2001). "The zone of inhibition was noted and interpreted using the Kirby–Bauer chart" (Wilker *et al.*,2005). Required dimensions of zone was calculated and compared with interpretative chart. The sensitivity of isolates towards antibiotics was calculated and classified as sensitive (S), intermediate (I) and resistant (R).

RESULTS AND DISCUSSION

The predominant colonies were isolated from Nutrient agar, EMB agar and MacConkey agar. The morphologies of microbial colonies are given in Table 1.

Above mentioned microbial organisms have been confirmed through microscopic examination, gram staining, motility and biochemical test shows following results:
a)Seventeen n Gram-positive organisms were isolated and they were identified as *Staphylococcus* spp. (Table2).
b)Thirty three Gram-negative organisms were isolated, two organisms were identified as *Salmonella* spp. Nineteen organisms were identified as *Escherichia* strains and twelve organisms were identified as *Pseudomonas* spp. (Table 3).

The identified pathogens were *Staphylococcus* spp., *E. coli* (strains), *Pseudomonas* spp., *Salmonella* spp.

Antibiotic Resistances

After identification, the isolated pathogens were tested for antibiotic resistance/susceptibility against commercially available antibiotics, using disc diffusion method, including: Cefazolin, Cefixime, Gatifloxacin, Enoxabid, Urixin, Levofloxacin, Ceftizoxime, Cefpirome, Erythrocin and Cefdinir. Results are shown in Tables 4, 5, 6 and 7.

Zone Break Point for *Staphylococcus*

The zone break point results for *Staphylococcus* are shown in Table 4a.
Most of the bacteria were found sensitive against antibiotics used in the study. I.E.S Staph-XV showed extreme resistance against Cefazolin, Cefixime, Gatifloxacin, Enoxabid, Urixin and is sensitive against the rest of the five antibiotics. I.E.S Staph-I, IV, VII, IX, X, XIII, XVI and XVII showed resistance against Cefixime and sensitive against the rest of the antibiotics.

According to a study, the*Staphylococcus*shows strong resistance against penicillin. This resistance is due to specificenzyme called β-lactamase (penicillinase) by formerly susceptible bacteria (Thind*et al.,* 2010).

Zone Break Point for *Escherichia*

The zone break point results for *Escherichia* are shown in Table 5a.Most of the *Escherichia* strains were found resistant specifically I.E.S *Escherichia*-XIII has shown extreme resistance against all antibiotics. I.E.S*Escherichia*-VI has shown extreme resistance against Cefazolin, Cefixime, Gatifloxacin, Enoxabid and Urixin. Cefixime were the least effective antibiotic against I.E.S*Escherichia*-XIII-XIX except I.E.S*Escherichia*-XIV, which showed extreme resistance against Enoxabid, Urixin, Levofloxacin and Erythrocin and very large zones of inhibition against Cefixime and Ceftizoxime.

A studyconducted in Bangladesh by Akond*et al.,* (2009) to examinethe antibiotic resistance of *E. coli*. In this study, they state that they ran 13 antimicrobial agents against fifty identified strains of *E. coli*. However, none of the strains showed resistance to gentamicin.

Mos*et al* (2010) conducted an investigation toassess"antibiotic sensitivity of *E. coli* strains isolated from numerous types of infected wounds. The results revealed a high sensitivity to amikacin and imipenem".

Zone Break Point for *Pseudomonas*

The zone break point results for*Pseudomonas* are shown in Table 6a.I.E.S*Ps-II* whichwas the only species showing the highest resistance against almost all of the used antibiotics. I.E.S*Ps*-I, III, IV, V, VII, VIII, IX and XII showed extreme resistance against Cefazolin and Cefixime.

An important investigation stated that *Pseudomonas aeruginosa* may grow in the presences of several drugs where other pathogens may not be able to live. This is an example of how antibiotic resistance may improve the virulence of such pathogens and make these bacteria able to survive in such niches. Therefore, antibiotic resistance might be considered as a virulence factor, particularly in the case of hospital settings where drug-resistant opportunistic bacteria may cause high morbidity and mortality" (Lye *et al.,* 2012).

Zone Break Point for *Salmonella*

The zone break point results for *Salmonella* are shown in Table 7.Both of the *Salmonella* spp. showed intermediate pattern and I.E.S*Salmonella-II* has shown extreme resistance against Ceftizoxime.

The microbial pollution at shorelinemostly arises due to untreated municipal sewage that is turn out from the urban area and is dumped at the coast water. It is pertinent to mention here that all the five locations selected for sampling were located in the vicinity of shipping ports. Further, during the course of study, it was observed that large number of people was living near the coastline whose occupation was only fishing. Therefore, fishing activities of these people and activities on cargo port may also have some impact on the pollution.

Table 1. Some morphological features of colonies observed on the different Medias.

Colonies feature on Nutrient agar	Colonies feature on MacConkey Agar	Colonies feature on EMB agar	Remarks
Colonies were large, thick, grayish white, moist, smooth, opaque or translucent discs. Some strains formed "mucoid" colonies	Mostly colonies were bright pink due to lactose fermentation	Colonies growth appears as large, blue-black colonies, often with a green metallic sheen	Characteristics feature of *Escherichia Coli* (strains).
Some strains grown on nutrient agar as smooth colonies, 2-4 mm in diameter.	Some colonies appeared colorless and transparent, though they sometimes have dark centers,	Colonies do not ferment lactose or produce acid, that's why these colonies appear grey	Characteristics feature of *Salmonella spp.*
Colonies appeared as greenish coloration due to production of Pyoverdin pigment	Mostly microbes were 2-3 mm, flat, smooth, non-lactose fermenting colonies with regular margin	-	Characteristics feature of *Pseudomonas* spp.
Most colonies appear relatively smooth, glossy and sometimes appearing wet. Colonies of most strains are usually opaque and may be pigmented white or cream and sometimes yellow to orange.	-	Usually form pinpoint colonies.	Characteristics feature of *Staphylococcus* spp.

Table 2. Biochemical Tests for Gram-positive organisms.

Isolates	Catalase	Coagulase	Organism
1	+	-	*Staphylococcus* sp.
2	+	-	*Staphylococcu*s sp.
3	+	-	*Staphylococcus* sp.
4	+	-	*Staphylococcus* sp.
5	+	-	*Staphylococcus* sp.
6	+	-	*Staphylococcus* sp.
7	+	-	*Staphylococcus* sp.
8	+	-	*Staphylococcus* sp.
9	+	-	*Staphylococcus*sp.
10	+	-	*Staphylococcus* sp.
11	+	-	*Staphylococcus* sp.
12	+	-	*Staphylococcus* sp.
13	+	-	*Staphylococcus* sp.
14	+	-	*Staphylococcus* sp.
15	+	-	*Staphylococcus* sp.
16	+	-	*Staphylococcus* sp.
17	+	-	*Staphylococcus* sp.

Table 3. Biochemical Tests for Gram-negative organisms.

Isolates	Oxidase	ONPG	CIT	GEL	LDC	ADH	ODC	H2S	URE	IND	VP	GLU	SUC	NIT	Organism
1.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
2.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
3.	-	+	-	-	+	-	-	-	-	+	-	+	-	+	*Escherichia* sp.
4.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
5.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
6.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
7.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
8.	-	+	-	-	+	-	-	-	-	+	-	+	-	+	*Escherichia* sp.
9.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
10.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
11.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
12.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
13.	-	+	-	-	+	-	-	-	-	+	-	+	-	+	*Escherichia* sp.
14.	-	+	-	-	+	-	-	-	-	+	-	+	-	+	*Escherichia* sp.
15.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
16.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
17.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
18.	-	-	-	-	+	-	+	-	-	+	-	+	+	+	*Escherichia* sp.
19.	-	+	-	-	+	-	-	-	-	+	-	+	-	+	*Escherichia* sp.
20.	+	+	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
21.	+	-	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
22.	+	-	+	+	+	+	-	-	+	-	-	+	-	-	*Pseudomonas* sp.
23.	+	-	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
24.	+	-	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
25.	+	+	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
26.	+	-	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
27.	+	+	+	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
28.	+	-	+	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
29.	+	-	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
30.	+	-	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
31.	+	+	-	-	-	-	-	-	-	-	-	+	-	-	*Pseudomonas* sp.
32.	-	-	+	-	+	-	-	+	-	-	-	+	-	+	*Salmonella* sp.
33.	-	-	+	-	+	-	-	+	-	-	-	+	-	+	*Salmonella* sp.

Table 4a. Zone break points for *Staphylococcus.*

Antibiotics	Disc (mcg)	Standard interpretation			Interpretation of zone diameters (mm)																				
					I.E.S *Staph*-I			I.E.S Staph-II			I.E.S *Staph*-III			I.E.S *Staph*-IV			I.E.S*Staph*-V			I.E.S*Staph*-VI			I.E.S*Staph*-VII		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Cefazolin	30	-	-	-	-	-	29	10	-	-	11	-	-	-	-	28	13	-	-	-	-	21	-	-	29
Cefixime	5	-	-	-	15	-	-	R	-	-	R	-	-	16	-	-	R	-	-	R	-	-	R	-	-
Gatifloxacin	5	≤19	-	≥20	-	-	39	-	-	33	-	-	25	-	-	31	-	-	21	-	-	29	-	-	30
Enoxabid	30	-	-	-	-	-	39	-	-	32	-	-	32	-	-	32	-	-	26	-	-	30	-	-	27
Urixin	50	-	-	-	-	-	30	-	-	22	18	-	-	-	-	18	17	-	-	14	-	-	13	-	-
Levofloxacin	5	-	-	-	-	-	34	-	-	30	-	-	25	-	-	30	-	-	26	-	-	26	-	-	27
Ceftizoxime	30	-	-	-	-	-	35	R	-	-	-	-	21	-	-	33	R	-	-	R	-	-	-	-	30
Cefpirome	30	-	-	-	-	-	26	9	-	-	16	-	-	-	-	20	11	-	-	18		-	-	-	26
Erythrocin	15	≤19	-	≥20	-	-	35	-	-	34	-	-	43	-	-	33	17	-	-	-	-	26	-	-	29
Cefdinir	5	-	-	-	-	-	32	15	-	-	-	-	20	-	-	30	11	-	-	19	-	-	-	-	30

R- Resistant, I- Intermediate, S- Sensitive.

Table 4b.Zone break points for *Staphylococcus.*

Antibiotics	Disc mcg)	Standard interpretation			Interpretation of zone diameters (mm)																				
					I.E.S *Staph*-VIII			I.E.S Staph-IX			I.E.S *Staph*-X			I.E.S *Staph*-XI			I.E.S *Staph*-XII			I.E.S *Staph*-XIII			I.E.S *Staph*-XIV		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Cefazolin	30	-	-	-	-	-	25	12	-	-	-	-	36	-	-	29	15	-	-	-	-	29	10	-	-
Cefixime	5	-	-	-	14	-	-	-	-	31	12	-	-	R	-	-	12	-	-	18	-	-	17	-	-
Gatifloxacin	5	≤19	-	≥20	-	-	23	-	-	32	-	-	29	-	-	21	-	-	28	-	-	31	-	-	38
Enoxabid	30	-	-	-	-	-	23	-	-	44	-	-	31	19	-	-	-	-	23	-	-	26	-	-	40
Urixin	50	-	-	-	17	-	-	-	-	30	18	-	-	18	-	-	-	-	27	-	-	24	-	-	30
Levofloxacin	5	-	-	-	-	-	27	-	-	40	-	-	29	-	-	28	-	-	32	-	-	30	-	-	23

Ceftizoxime	30	-	-	-	-	-	32	-	-	50	-	-	21	17	-	-	20	-	-	-	-	21	10	-	-
Cefpirome	30	-	-	-	18	-	-	-	-	40	-	-	24	-	-	28	-	-	20	15	-	-	15	-	-
Erythrocin	15	≤19	-	≥20	-	-	30	R	-	-	-	-	36	9	-	-	19	-	-	R	-	-	-	-	35
Cefdinir	5	-	-	-	-	-	35	-	-	36	-	-	27	-	-	23	10	-	-	-	-	26	-	-	29

Table 4c, Zone break points for *Staphylococcus.*

Antibiotics	Disc content (mcg)	Interpretation of zone diameters (mm)											
		Standard interpretation			I.E.S *Staph*-XV			I.E.S *Staph*-XVI			I.E.S *Staph*-XVII		
		R	I	S	R	I	S	R	I	S	R	I	S
Cefazolin	30	-	-	-	R	-	-	-	-	35	-	-	36
Cefixime	5	-	-	-	R	-	-	R	-	-	R	-	-
Gatifloxacin	5	≤19	-	≥20	R	-	-	-	-	35	-	-	44
Enoxabid	30	-	-	-	R	-	-	-	-	21	-	-	28
Urixin	50	-	-	-	R	-	-	18	-	-	-	-	40
Levofloxacin	5	-	-	-	-	-	40	-	-	31	-	-	42
Ceftizoxime	30	-	-	-	-	-	36	-	-	27	R	-	-
Cefpirome	30	-	-	-	-	-	42	-	-	31	-	-	26
Erythrocin	15	≤19	-	≥20	R	-	-	-	-	25	-	-	48
Cefdinir	5	-	-	-	-	-	34	-	-	32	-	-	26

R- Resistant, I- Intermediate, S- Sensitive.

Table 5a, Zone break points for *Escherichia.*

Antibiotics	Disc content (mcg)	Interpretation of zone diameters (mm)					
		I.E.S Escherichia-I	I.E.S Escherichia –II	I.E.S*Escherichia* –III	I.E.S Escherichia -IV	I.E.S Escherichia -V	I.E.S Escherichia –VI
Cefazolin	30	18	19	22	R	R	R
Cefixime	5	23	21	22	R	R	R
Gatifloxacin	5	10	29	16	13	9	R
Enoxabid	30	R	29	R	R	R	R
Urixin	50	R	20	R	R	R	R
Levofloxacin	5	8	28	15	11	R	14
Ceftizoxime	30	33	31	34	16	14	11
Cefpirome	30	27	26	29	R	R	10
Erythrocin	15	R	R	R	R	R	11
Cefdinir	5	20	21	23	R	R	10

R- Resistant

Table 5b, Zone break points for *Escherichia.*

		Interpretation of zone diameters (mm)					
Antibiotics	Disc content (mcg)	I.E.S *Escherichia* -VII	I.E.S *Escherichia* –VIII	I.E.S Escherichia –IX	I.E.S Escherichia -X	I.E.S Escherichia –XI	I.E.S *Escherichia* -XII
Cefazolin	30	17	R	20	24	02	R
Cefixime	5	20	R	23	28	24	R
Gatifloxacin	5	12	42	24	34	24	12
Enoxabid	30	R	30	27	40	30	R
Urixin	50	R	24	23	32	30	R
Levofloxacin	5	11	R	3	39	20	11
Ceftizoxime	30	31	16	38	39	R	11
Cefpirome	30	25	R	30	30	14	R
Erythrocin	15	R	R	27	14	R	R
Cefdinir	5	21	R	25	26	16	R

R- Resistant

Table 5c, Zone break points for *Escherichia.*

		Interpretation of zone diameters (mm)						
Antibiotics	Disc content (mcg)	I.E.S *E.coli*-XIII	I.E.S *E.coli*-XIV	I.E.S *E.coli*-XV	I.E.S *E.coli*-XVI	I.E.S *E.coli*-XVII	I.E.S *E.coli*-XVIII	I.E.S *E.coli*-XIX
Cefazolin	30	R	22	27	R	20	R	10
Cefixime	5	R	30	R	R	R	R	R
Gatifloxacin	5	R	11	12	18	24	12	21
Enoxabid	30	R	R	30	19	26	R	22
Urixin	50	R	R	R	15	20	R	R
Levofloxacin	5	R	R	26	17	27	11	16
Ceftizoxime	30	R	40	15	12	11	11	16
Cefpirome	30	R	26	16	15	11	R	11
Erythrocin	15	R	R	30	20	20	R	10
Cefdinir	5	R	26	20	R	10	R	30

Table 6a, Zone break points for *Pseudomonas.*

					Interpretation of zone diameters (mm)																	
Antibiotics	Disc content (mcg)	Standard interpretation			I.E.S*Ps*-I			I.E.S*Ps*-II			I.E.S*Ps*-III			I.E.S*Ps*-IV			I.E.S*Ps*-V			I.E.S*Ps*-VI		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Cefazolin	30	-	-	-	R	-	-	R	-	-	R	-	-	R	-	-	R	-	-	-	-	30
Cefixime	5	-	-	-	R	-	-	R	-	-	R	-	-	R	-	-	R	-	-	R	-	-
Gatifloxacin	5	≤ 19	-	≥ 20	-	-	25	R	-	-	-	-	25	-	-	25	-	-	31	-	-	34
Enoxabid	30	-	-	-	-	-	39	R	-	-	-	-	36	-	-	33	-	-	39	-	-	40
Urixin	50	-	-	-	-	-	21	R	-	-	-	-	21	-	-	17	-	-	28	-	-	26
Levofloxacin	5	≤ 17	-	≥ 18	-	-	22	15	-	-	-	-	29	-	-	23	-	-	32	13	-	-
Ceftizoxime	30	-	-	-	r	-	-	16	-	-	12	-	-	R	-	-	11	-	-	R	-	-
Cefpirome	30	≤ 19	20-24	≥ 25	14	-	-	R	-	-	16	-	-	12	-	-	12	-	-	12		-
Erythrocin	15	-	-	-	R	-	-	R	-	-	14	-	-	-	-	20	-	-	19	R	-	-
Cefdinir	5	-	-	-	R	-	-	R	-	-	12	-	-	13	-	-	R	-	-	R	-	-

R- Resistant, I- Intermediate, S- Sensitive

Table 6b.Zone break points for *Pseudomonas.*

Antibiotics	Disc content (mcg)	Standard interpretation			Interpretation of zone diameters (mm)																	
					I.E.S*Ps*-VII			I.E.S*Ps*-VIII			I.E.S*Ps*-IX			I.E.S*Ps*-X			I.E.S*Ps*-XI			I.E.S*Ps*-XII		
		R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Cefazolin	30	-	-	-	R	-	-	R	-	-	R	-	-	R	-	-	10	-	-	R	-	-
Cefixime	5	-	-	-	R	-	-	R	-	-	R	-	-	R	-	-	18	-	-	R	-	-
Gatifloxacin	5	≤19	-	≥20	-	-	25	-	-	40	-	-	24	13	-	-	-	-	31	-	-	29
Enoxabid	30	-	-	-	-	-	36	-	-	45	-	-	20	16	-	-	-	-	31	-	-	33
Urixin	50	-	-	-	-	-	21	-	-	20	-	-	24	-	-	19	19	-	-	-	-	23
Levofloxacin	5	-	-	-	-	-	29	-	-	22	-	-	26	-	-	26	-	-	34	-	-	34
Ceftizoxime	30	-	-	-	12	-	-	-	-	20	R	-	-	12	-	-	R	-	-	13	-	-
Cefpirome	30	-	-	-	16	-	-	13	-	-	13	-	-	18	-	-	21	-	-	16	-	-
Erythrocin	15	≤19	-	≥20	14	-	-	-	-	30	R	-	-	R	-	-	-	-	34	R	-	-
Cefdinir	5	-	-	-	12	-	-	15	-	-	-	-	28	-	-	23	-	-	25	R	-	-

R- Resistant, I- Intermediate, S- Sensitive

Table 7.Zone break points for *Salmonella.*

Antibiotics	Disc content (mcg)	Interpretation of zone diameters (mm)	
		I.E.S *Salmonella*-I	I.E.S *Salmonella*-II
Cefazolin	30	19	15
Cefixime	5	33	24
Gatifloxacin	5	24	28
Enoxabid	30	16	22
Urixin	50	20	21
Levofloxacin	5	40	30
Ceftizoxime	30	09	R
Cefpirome	30	12	11
Erythrocin	15	29	31
Cefdinir	5	17	15

R- Resistant, I- Intermediate, S- Sensitive

CONCLUSION

The occurrence of antibiotic-resistant bacteria in aquatic environments has increased significantly because of extensive exposure of antibiotics by humans. The main reason is the selection for resistant strains and the ability of such strains to exchange plasmids encoding resistance (Baya *et al.*, 1986). It is pertinent to mention here that "bacteria can transfer resistance plasmids *in situ* to indigenous micro flora" (Mach and Grimes, 1982).

Grimes *et al*., (1984) carried out an inquiry to prove that the changes which occurs in bacterial structure, species composition and community is only because of excessive throwing of huge quantities of pharmaceutical, chemical and domestic wastes into the ocean.

The point of concern is that the indiscriminate disposal of waste water is deteriorating the marine ecosystem. The present investigation also reveals the survival of antibiotic resistant organisms in the marine environment. The study indicated that these microorganisms may have the ability to transfer their antibiotic resistance which may even resist third and fourth generation antibiotics.

REFERENCES

Akond, M.A., S. Alam, S.M.R. Hassan and M. Shirin (2009). Antibiotic resistance of E coli isolated from poultry and poultry environment of Bangladesh. *Internet J Food Saf.*, 11: 19–23.

Bauer A.W., W.M. Kirby, J.C. Sherris and M. Turck (1966).Antibiotic susceptibility testing by a standardized single disk method.*Am. J ClinPathol.*, 45: 493-6.

Baya, A.M., Brayton, P.R., Brown, V.L., Grimes, D.J., Russek-Cohen E. and Colwell R.R. (1986). Coincident Plasmids and Antimicrobial Resistance in Marine Bacteria Isolated from Polluted and Unpolluted Atlantic Ocean Samples. *Applied and Environmental Microbiology*, 51: 1285-1292.

Cosgrove, S. E. (2006). The relationship between antimicrobial resistance and patient outcomes: Mortality, length of hospital stay, and health care costs. *Clinical Infectious Diseases*: S82–S89.doi:10.1086/499406.

Davies J. and D. Davies (2010).Origins and evolution of antibiotic resistance.*Microbiol. Mol. Biol. Re.,* 74: 417–433.

David F. and D.F.J. Brown (2001).Instrumentation in antimicrobial susceptibility testing. *Journal of Antimicrobial Chemotherapy*, 48 (Suppl): 81-85.

Grimes, D.J., F.L. Singleton and R.R. Colwell (1984).Allogenic succession of marine bacterial communities in response to pharmaceutical waste.*J. Appl. Bacteriol.*, 57: 247-261.

Greig, J., A. Rajic, I. Young, M. Mascarenhas, L. Waddell and J. LeJeune (2015). A scoping review of the role of wildlife in the transmission of bacterial pathogens and antimicrobial resistance to the food chain. *Zoonoses Public Health, 62: 269-284.*

Hendleyand J.O. (1997). The problem of Antibiotic Resistance.*Virginia Medical Quarterly*, 124: 19-24.

Lye D.C., A. Earnest , M.L. Ling, T.E. Lee, H.C. Yong, D.A. Fisher, P. Krishnan and L.Y. Hsu (2012).The impact of multidrug resistance in healthcare-associated and nosocomial Gram-negative bacteraemia on mortality and length of stay: cohort study. *Clin.Microbiol. Infect.* 18: 502-8.

Mach, P.A. and D.J. Grimes (1982). R-plasmid transfer in a wastewater treatment plant. *Appl. Environ. Microbiol*., 44: 1395-1403.

Mos I., O. Micle, M. Zdranca, M. Muresan and L. Vicas (2010). Antibiotic sensitivity of the *E. coli* strains isolated from infected skin wounds.*Farmacia*, 58: 637–45.

Thind P., S.K. Prakash, A.Wadhwa, V.K. Garg and B. Pati (2010).Bacteriological profile of community-acquired pyodermas with special reference to methicillin resistant *Staphylococcus aureus*.*Indian J DermatolVenereolLeprol*, 76: 572-4.

Wilker M.A., F.R. Cockerill and W.A. Craig (2005).*Performance standards for anti-microbial susceptibility testing*.Vol. 25. Wayne, PA: CLSI; 15 th informational supplement; pp. M100–SI5.

www.health.fgov.be/WHI3/periodical/months/wwhv2n5tekst/WWH2306984.htm

(*Accepted for publication November 2020*)