Bangladesh J Microbiol, Volume 35, Number 2, December 2018, pp 108-114

Original Article

Influence of multi-species biofilm formed in vitro from different environmental samples on the drug-resistance traits of resident bacteria

Saurab Kishore Munshi[1], Tamanna Haque[1] and Rashed Noor[2]*

[1]Department of Microbiology, Stamford University Bangladesh, 51 Siddeswari Road, Dhaka-1217

[2]School of Life Sciences (SLS), Independent University, Bangladesh (IUB), Plot 16, Block B, Aftabuddin Ahmed Road, Bashundhara, Dhaka 1229, Bangladesh

Present study was designed to investigate whether the formation of multi-species biofilm impart any effect on the enhancement of the antibiotic resistance of the planktonic cells residing in the general nutrient enriched environments. In this regard, surface water of pond, mud from agricultural field, pharmaceutical waste waters, municipal waste water, hospital waste water and the domestic waste samples were collected and were induced to form an *in vitro* multi-species biofilm. All samples were found to contain huge array of pathogenic bacteria. The pathogenic isolates both from the bulk samples and their corresponding biofilms were subjected to antibiogram assay using 13 commonly used antibiotics. An extended frequency of drug resistance was observed in case of biofilm isolates. The findings of present study revealed that a number of bacterial isolates which showed sensitivity towards antibiotics, acquired drug-resistance when they were isolated from biofilms.

Key words: Multi-species biofilm, drug resistance, surface water, waste water

Introduction

Antibiotic resistance has been a global interference in disease medication principally shaded by the pathogenic microorganisms, especially in the developing countries with high density of population under the line of poverty as in Bangladesh[1-9]. Unethical usage of non-prescribed antibiotics and clinically unsafe practice of self-medication led to the development of the unsolicited resistance by the infecting bacteria[2,5,10]. Several studies in Bangladesh found extended numbers of drug resistant isolates including the multi-drug resistant (MDR) ones in different food and water samples, analytical of their possible propagation into the natural environments[5,11-15]. The mechanism of bacterial drug-resistance is usually known to be based on the production of inactivating enzymes, change in the drug-target sites, and the exclusion of the antibiotic from the target site[16]. Even the efficiency of the prescribed antibiotic may be threatened by genetic mutation and the possible spread of the drug-resistant genes through horizontal or vertical transmission, or through the extra-chromosomal plasmid genes, and finally by the transposons[2,17,18].

Majority of bacteria in natural habitats, especially in natural aquatic ecosystems are organized in biofilms, simply defined as microbial communities that are attached to a surface and embedded in a self-produced matrix composed of extracellular polymeric substances[19-21]. Although single-species biofilms exist in a variety of infections and on the surface of medical implants, multi-species biofilms may also predominate in the natural environments[21-23]. The multi-species biofilms may indeed offer heightened opportunities for interactions such as horizontal gene transfer and co-metabolism in natural environments which may take part in the elevated drug resistance.

Most of the studies concerning drug resistance in the environmental samples have focused on the bulk sample and situation of individual bacteria isolated from that samples[5,11-15]. However, those studies did not focus the situation in case of biofilms, which is widely known to be the preferred pattern of life of many bacteria regarding bacterial survival in the adverse environment. High bacterial density and diversity are commonly observed in biofilms from wastewater systems as well as from surface water and drinking water distribution systems where antibiotic resistance may frequently spread[18,24,25]. Considering all these facts, present study endeavored to estimate the possible occurrence of the enhanced drug resistance of bacterial isolates due to their participation in the formation of the multi-species biofilms from surface water and different waste water samples.

Materials and methods

Study period and sampling

The study was carried out at the Microbiology Laboratory, Stamford University Bangladesh from August 2016 to October 2016. Different nutrient rich aqueous samples such as surface water of pond, mud from agricultural land, municipal waste water, pharmaceutical waste water, domestic waste and hospital waste water were randomly collected in sterile PET bottle or jar from different locality of Dhaka city and transported to the laboratory at the earliest convenient. For the isolation and enumeration of

***Corresponding author**:
Dr. Rashed Noor, Associate Professor, School of Life Sciences (SLS),Independent University, Bangladesh (IUB), Plot 16, Block B, Aftabuddin Ahmed Road, Bashundhara, Dhaka 1229, Bangladesh. E- mail: noor.rashed@yahoo.com

pathogenic bacteria, 10 ml or g of each sample was homogenized in 90 ml normal saline and diluted up to 10^{-5} according to the standard guideline[13-15].

Estimation of total viable bacteria, Escherichia coli, Klebsiella spp., Staphylococcus spp. and Bacillus spp.

From the dilutions 10^{-3} and 10^{-5}, 0.1 ml of each sample was spread onto the nutrient agar (NA) media for the enumeration of total viable bacteria. Likewise, 0.1 ml of each sample from the raw suspension and dilution 10^{-2} were introduced onto MacConkey agar, mannitol salt agar (MSA), mannitol egg yolk polymyxin (MYP) agar and cetrimide agar for the isolation of coliforms (*Escherichia coli* and *Klebsiella* spp.), *Staphylococcus* spp., *Bacillus* spp. and *Pseudomonas* spp., consecutively. All the plates were then incubated at 37 °C for 24 hours[13-15].

Isolation of Salmonella spp., Shigella spp. and Vibrio spp.

By considering the possible occurrence of viable but non-culturable (VBNC) cells[15,27-29], 10 ml of sample was transferred into 90 ml of selenite cysteine broth (SCB) and alkaline peptone water (APW) for the enrichment of *Salmonella*, *Shigella*, and *Vibrio* spp., respectively and incubated at 37 °C for 6 hours. After incubation, the samples were diluted up to 10^{-4} and then 0.1 ml of samples from each of the 10^{-2} and 10^{-4} dilutions were spread onto Salmonella-Shigella (SS) agar and thiosulfate citrate bile salt sucrose (TCBS) agar for the isolation of *Salmonella* spp. & *Shigella* spp., and *Vibrio* spp., consecutively.

Biochemical identification of the bacterial isolates

Finally, all the isolates were biochemically examined for their identification following standard procedures as described earlier[12,15,26,30].

In vitro biofilm formation and recovery of the planktonic cells

On the other hand, 10 ml or g of each sample was introduced and homogenized in 90 ml Luria-Bertani (LB) broth soon after the arrival of the samples and then incubated at 37 °C with constant shaking at 150 rotations per minute (rpm) for three to seven days. Planktonic (free-living) bacteria residing in each sample was allowed to grow in the broth medium until nutrient was declined which was the selective pressure for biofilm formation. As a consequence, drop in planktonic bacterial cell number would result in the formation of biofilm. Macroscopic flocs of thin opaque layer suspended surrounding the surface of the medium anchoring flask was the indicative of biofilm formation[31].

The planktonic cells were further recovered from the biofilm by isolating them on the specific media. One loopful culture from the biofilm of each sample, which was collected very carefully from the surface of the broth medium, was inoculated onto MacConkey agar, MSA agar, MYP agar and cetrimide agar for the isolation of coliforms (*Escherichia coli* and *Klebsiella* spp.), *Staphylococcus* spp., *Bacillus* spp. and *Pseudomonas* spp., consecutively. Isolation of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. was carried out following the same procedure as described previously[13-15].

Antibiotic susceptibility test of the isolates

The standard agar-disc-diffusion method (Kirby Bauer technique) was used to examine the antibiotic susceptibility of the pathogenic bacteria isolated directly from the samples as well as from the biofilm formed from the same samples on Mueller-Hinton agar (Difco, Detroit, MI) with same set of antibiotics[11,15,32,33]. Antibiotics used in this study included trimethoprime/sulfamethoxazole (25 µg), erythromycin (15 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), cefixime (5 µg), polymyxin B (300 units), imepenem (10 ¼g), gentamicin (10 µg), nalidixic acid (30 µg) and azithromycin (15 µg).

Results and discussion

Unregulated disposal of industrial, hospital and domestic wastes containing organic substances along with toxic chemicals including antibiotics as well as pathogenic bacteria to the environment may play a huge role in the accumulation of bacterial drug-resistance[18,34-39]. As stated elsewhere, most of the studies in Bangladesh concerning antibiotic resistance of bacteria and their distribution in the environment are based on the analysis of bulk water, food samples and patients' derived isolates so far[2,4,5,11-15]. To add a new dimension to this area of research, multi-species biofilms, which are commonly formed in the environment by the close interaction of bacteria, were analyzed in this study.

Determination of the presence of pathogenic bacteria from the environmental samples tested

All the samples were found to contain huge load of viable bacteria in a range of 2.3×10^5 - 1×10^8 cfu/ml or g (Table 1) as found previously from such environmental samples[40,11-14]. Pathogenic bacteria were also recovered in significant quantities from all the samples, especially surface water sample contained all the pathogenic bacteria tested except *Shigella* spp. which was actually absent in all samples. *Klebsiella* spp., *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. were found to be present in all samples in the average of 10^3, 10^4, 10^3 and 10^3 cfu/ml or g, consecutively (Table 1). *E. coli* was absent in pharmaceutical and hospital waste water samples but resided in the other four samples within the range of 2.5×10^3 - 3.3×10^4 cfu/ml or g. *Salmonella* spp. was isolated from surface water, municipal waste water and domestic waste samples with average load of 10^4 cfu/ml or g (Table 1). Only surface water sample exhibited the presence of *Vibrio* spp (1.3×10^5 cfu/ml). Interestingly, pathogenic bacteria which were isolated from the tested environmental samples were also recovered from the biofilms of the samples.

Enhanced antibiotic resistance of the isolates recovered from biofilms

In agreement with the previous studies[18,41,42], the result of antibiogram assay for all the samples apparently showed that

Table 1: *Isolation and quantification of pathogenic bacteria from different environmental samples.*

Sample	TVB (cfu/ml or g)	*E. coli* (cfu/ml or g)	*Klebsiella* spp (cfu/ml or g)	**Salmonella* spp. (cfu/ml or g)	*Pseudomonas* spp. (cfu/ml or g)	*Bacillus* spp (cfu/ml or g)	**Vibrio* spp. (cfu/ml or g)	*Staphylococcus* spp. (cfu/ml or g)
Surface water	1.2×10^{7}	3.3×10^{4}	3.4×10^{3}	3.0×10^{3}	3.0×10^{4}	2.3×10^{3}	1.3×10^{3}	3.0×10^{5}
Mud	2.3×10^{5}	2.5×10^{3}	1.5×10^{3}	0	2.0×10^{3}	2.9×10^{3}	0	2.0×10^{4}
Municipal waste water	1.5×10^{8}	2.8×10^{3}	2.9×10^{3}	3.8×10^{4}	2.1×10^{3}	3.8×10^{3}	0	2.2×10^{5}
Pharmaceutical waste water	3.3×10^{6}	0	2.8×10^{3}	0	3.9×10^{3}	2.0×10^{3}	0	1.1×10^{3}
Hospital waste water	2.7×10^{6}	0	1.4×10^{4}	0	1.0×10^{3}	1.5×10^{3}	0	3.1×10^{4}
Domestic waste	4.0×10^{7}	2.2×10^{4}	4.3×10^{3}	2.2×10^{4}	2.2×10^{3}	2.0×10^{3}	0	2.9×10^{5}

TVB- Total viable bacteria
The experiments were in triplicates. Average count (cfu/ml or g) from all samples have been shown here.
*Bacterial load after enrichment (Prior to enrichment, the recovery was nil).
Shigella spp. was absent in all samples.

those bacterial isolates which were found to be sensitive against any of the antibiotics used, exhibited resistance against that antibiotic in almost all instances when they were isolated from biofilms (Tables 2-7). The only exception was gentamicin ((GEN, 10 µg), against which none of the isolates showed resistance whether they were from bulk samples or from biofilms (Tables 2-7). In cohort to the previous studies on surface water and wastes[11,12,40,43], present study recovered a significant number of multidrug resistant isolates. The frequency of MDR cases was exceedingly accelerated when the bacteria were isolated from biofilm. If the samples were more specifically assessed, the isolates from surface water sample which exhibited sensitivity against trimethoprim-sulfamethoxazole (TRI-SUL, 25 µg), erythromycin (ERY, 15 µg), ceftriaxone (CEF, (30 µg), ampicillin (AMP, 10 µg), polimyxin B (POL-B, 300 units) and nalidixic acid (NAL, 30 µg) gained resistance when they were allowed to grow in biofilm (Table 2). A few of the isolates were found to keep their sensitivity against some antibiotics. *Klebsiella* spp. remained to be sensitive against tetracycline (TET, 30 µg) and cefixime (CFX, 5 µg) besides GEN. *Pseudomonas* spp. retained sensitivity against CFX, imepenem (IPM, 10 µg) and azithromycin (AZI, 15 µg). The sensitivity of *Vibrio* spp. remained to be unchanged against chloramphenicol (CHO, 30 µg) and ciprofloxacin (CIP, 5 µg). *Salmonella* spp. retained sensitivity against TET (Table 2).

In the mud sample, all the sensitive isolates acquired resistance against TRI-SUL, CEF, CIP, AMP, TET, CHO, POL-B and NAL (Table 3). *Klebsiella* spp. remained sensitive against CFX and AZI, whereas *Pseudomonas* spp. stayed sensitive against IPM

Table 2: *Antibiotics susceptibility pattern of the isolates from surface water*

	Antibiotics Isolates	TRI-SUL	ERY	CEF	CIP	AMP	TET	CHO	CFX	POL-B	IPM	GEN	NAL	AZI
Bulk sample	*E. coli*	S	S	ND	S	R	R	S	S	ND	S	S	S	S
	Klebsiella spp.	S	S	ND	R	S	S	R	S	ND	S	S	R	S
	Salmonella spp.	R	S	S	S	S	S	S	ND	ND	S	S	S	ND
	Pseudomonas spp.	S	ND	S	S	S	S	S	S	ND	S	S	R	S
	Bacillus spp.	S	S	ND	S	R	S	S	ND	S	S	S	S	ND
	Vibrio spp.	R	S	ND	S	R	R	R	S	R	R	S	ND	S
	Staphylococcus spp.	S	S	S	S	S	S	S	ND	R	ND	S	ND	S
Biofilm	*E. coli*	R	R	ND	R	R	R	R	R	ND	R	S	R	R
	Klebsiella spp.	R	R	ND	R	R	S	R	S	ND	R	S	R	R
	Salmonella spp.	R	R	R	R	R	S	R	ND	ND	R	S	R	ND
	Pseudomonas spp.	R	ND	R	R	R	R	R	S	ND	S	S	R	S
	Bacillus spp.	R	R	ND	ND	R	R	R	ND	R	R	S	R	ND
	Vibrio spp.	R	R	ND	S	R	R	S	R	R	R	S	ND	R
	Staphylococcus spp.	R	R	R	R	R	R	R	ND	R	ND	S	ND	R

ND: Not done; R: Resistant; S: Sensitive
TRI-SUL= Trimethoprime/sulfamethoxazole (25 µg); ERY= Erythromycin (15 µg); CEF= Ceftriaxone (30 µg); CIP= Ciprofloxacin (5 µg); AMP= Ampicillin (10 µg), TET= Tetracycline (30 µg), CHO= Chloramphenicol (30 µg), CFX= Cefixime (5 µg), POL-B= Polymyxin B (300 units), IPM= Imepenem (10 ¼g), GEN= Gentamicin (10 µg), NAL= Nalidixic acid (30 µg); AZI= Azithromycin (15 µg).

Table 3: *Antibiotics susceptibility pattern of the isolates from mud*

	AntibioticsIsolates	TRI-SUL	ERY	CEF	CIP	AMP	TET	CHO	CFX	POL-B	IPM	GEN	NAL	AZI
Bulk sample	*E. coli*	R	S	ND	S	S	R	S	S	ND	R	S	R	S
	Klebsiella spp.	S	S	ND	R	S	S	S	S	ND	R	S	R	S
	Pseudomonas spp.	S	ND	S	S	S	S	S	S	ND	S	S	R	S
	Bacillus spp.	S	S	ND	S	R	S	S	ND	S	S	S	S	ND
	Staphylococcus spp.	S	S	S	S	S	S	S	ND	R	ND	S	ND	S
Biofilm	*E. coli*	R	R	ND	R	R	R	R	R	ND	R	S	R	R
	Klebsiella spp.	R	R	ND	R	R	R	R	S	ND	R	S	R	S
	Pseudomonas spp.	R	ND	R	R	R	R	R	S	ND	S	S	R	S
	Bacillus spp.	R	R	ND	R	R	R	R	ND	R	R	S	R	ND
	Staphylococcus spp.	R	S	R	R	R	R	R	ND	R	ND	S	ND	R

ND: Not done; R: Resistant; S: Sensitive
TRI-SUL= Trimethoprime/sulfamethoxazole (25 µg); ERY= Erythromycin (15 µg); CEF= Ceftriaxone (30 µg); CIP= Ciprofloxacin (5 µg); AMP= Ampicillin (10 µg), TET= Tetracycline (30 µg), CHO= Chloramphenicol (30 µg), CFX= Cefixime (5 µg), POL-B= Polymyxin B (300 units), IPM= Imepenem (10 ¼g), GEN= Gentamicin (10 µg), NAL= Nalidixic acid (30 µg); AZI= Azithromycin (15 µg).

along with these two antibiotics. The sensitivity of *Staphylococcus* spp. persisted against ERY (Table 3). In the municipal waste sample, all the sensitive isolates were found to be resistant against AMP, CHO, POL-B and NAL afterwards (Table 4). Almost all the isolates with one exception gained resistance against TRI-SUL, CIP, TET, ERY, and CEF (Table 4). Interestingly, the sensitive isolates retained their sensitivity against CFX. Two of the isolates remained sensitive each against IPM and AZI (Table 4).

In the pharmaceutical waste sample, all the sensitive isolates showed resistance against TRI-SUL, CEF, CIP, AMP, TET, CHO, POL-B and NAL when they were isolated from biofilm (Table 5). *Bacillus* spp. and *Staphylococcus* spp. which were found to be sensitive towards almost all antibiotics, acquired resistance against almost all of them afterwards and so was observed in other samples also (Tables 2-7). *Pseudomonas* spp. retained their sensitivity against CFX, IPM and AZI (Table 5). In hospital waste water samples, the sensitive isolates attained resistance against TRI-SUL, ERY, CEF, CIP, AMP, TET, CHO, POL-B and NAL (Table 6). As found in the other two samples, the sensitivity of the isolates remained to be unchanged against CFX. In addition to this, *Pseudomonas* spp. retained their sensitivity against IPM (Table 6). In the domestic waste sample, against AMP and POL-B, all the sensitive isolates gained resistance afterwards (Table 7). Almost similar findings were observed against TRI-SUL, ERY, CEF, CIP, TET, CHO and NAL with the retention of a sensitive isolate. Two isolates in each instance retained sensitivity against IPM, CFX and AZI (Table 7).

The enhanced antibiotic resistance might be due to several factors including the limited diffusion through the biofilm, enzyme mediated resistance, interaction and neutralization of the antimicrobial substance by the biofilm, metabolic state of the organisms in the biofilm, genetic adaptation, outer membrane structure and efflux pump[44]. The data of the present study suggested that multispecies biofilm may actively take part in the frequent spreading of antibiotic resistance gene in the natural

Table 4: *Antibiotics susceptibility pattern of the isolates from municipal waste water*

AntibioticsIsolates		TRI-SUL	ERY	CEF	CIP	AMP	TET	CHO	CFX	POL-B	IPM	GEN	NAL	AZI
Bulk sample	*E. coli*	R	S	ND	S	S	R	S	S	ND	R	S	R	S
	Klebsiella spp.	S	S	ND	R	S	R	S	S	ND	R	S	R	S
	Salmonella spp.	R	S	S	R	S	R	S	ND	ND	S	S	S	ND
	Pseudomonas spp.	S	ND	S	S	S	S	S	S	ND	S	S	R	S
	Bacillus spp.	S	S	S	ND	R	S	S	ND	S	S	S	S	ND
	Staphylococcus spp.	S	S	S	S	S	S	S	ND	R	ND	S	ND	S
Biofilm	*E. coli*	R	S	ND	R	R	R	R	S	ND	S	S	R	R
	Klebsiella spp.	R	R	ND	R	R	R	R	S	ND	R	S	R	S
	Salmonella spp.	R	R	R	R	R	R	R	ND	ND	R	S	R	ND
	Pseudomonas spp.	S	ND	R	R	R	R	R	S	ND	S	S	R	S
	Bacillus spp.	R	R	R	ND	R	R	R	ND	R	R	S	R	ND
	Staphylococcus spp.	R	R	S	S	R	S	R	ND	R	ND	S	ND	R

ND: Not done; R: Resistant; S: Sensitive
TRI-SUL= Trimethoprime/sulfamethoxazole (25 µg); ERY= Erythromycin (15 µg); CEF= Ceftriaxone (30 µg); CIP= Ciprofloxacin (5 µg); AMP= Ampicillin (10 µg), TET= Tetracycline (30 µg), CHO= Chloramphenicol (30 µg), CFX= Cefixime (5 µg), POL-B= Polymyxin B (300 units), IPM= Imepenem (10 ¼g), GEN= Gentamicin (10 µg), NAL= Nalidixic acid (30 µg); AZI= Azithromycin (15 µg).

Table 5: *Antibiotics susceptibility pattern of the isolates from pharmaceutical waste water*

AntibioticsIsolates		TRI-SUL	ERY	CEF	CIP	AMP	TET	CHO	CFX	POL-B	IPM	GEN	NAL	AZI
Bulk sample	*Klebsiella* spp.	R	S	ND	R	R	R	S	S	ND	S	S	R	S
	Pseudomonas spp.	S	ND	S	S	S	S	S	S	ND	S	S	R	S
	Bacillus spp.	S	S	ND	S	S	S	S	ND	R	S	S	S	ND
	Staphylococcus spp.	S	R	S	S	S	S	S	ND	S	ND	S	ND	R
Biofilm	*Klebsiella* spp.	R	R	ND	R	R	R	R	S	ND	R	S	R	S
	Pseudomonas spp.	R	ND	R	R	R	R	R	S	ND	S	S	R	S
	Bacillus spp.	R	R	ND	R	R	R	R	ND	R	R	S	R	ND
	Staphylococcus spp.	R	S	R	R	R	R	R	ND	R	ND	S	ND	R

ND: Not done; R: Resistant; S: Sensitive
TRI-SUL= Trimethoprime/sulfamethoxazole (25 µg); ERY= Erythromycin (15 µg); CEF= Ceftriaxone (30 µg); CIP= Ciprofloxacin (5 µg); AMP= Ampicillin (10 µg), TET= Tetracycline (30 µg), CHO= Chloramphenicol (30 µg), CFX= Cefixime (5 µg), POL-B= Polymyxin B (300 units), IPM= Imepenem (10 ¼g), GEN= Gentamicin (10 µg), NAL= Nalidixic acid (30 µg); AZI= Azithromycin (15 µg).

Table 6: *Antibiotics susceptibility pattern of the isolates from hospital waste water*

AntibioticsIsolates		TRI-SUL	ERY	CEF	CIP	AMP	TET	CHO	CFX	POL-B	IPM	GEN	NAL	AZY
Bulk sample	*Klebsiella* spp.	S	S	ND	R	S	S	R	S	ND	R	S	S	S
	Pseudomonas spp.	ND	ND	S	S	S	S	S	S	ND	S	S	R	S
	Bacillus spp.	S	S	ND	S	R	S	S	ND	S	S	S	S	ND
	Staphylococcus spp.	S	S	S	S	S	S	S	ND	R	ND	S	ND	S
Biofilm	*Klebsiella* spp.	R	R	ND	R	R	R	R	S	ND	R	S	R	R
	Pseudomonas spp.	ND	ND	R	R	R	R	R	S	ND	S	S	R	S
	Bacillus spp.	R	R	ND	R	R	R	R	ND	R	R	S	R	ND
	Staphylococcus spp.	R	R	R	R	R	R	R	ND	R	ND	S	ND	R

ND: Not done; R: Resistant; S: Sensitive
TRI-SUL= Trimethoprime/sulfamethoxazole (25 µg); ERY= Erythromycin (15 µg); CEF= Ceftriaxone (30 µg); CIP= Ciprofloxacin (5 µg); AMP= Ampicillin (10 µg), TET= Tetracycline (30 µg), CHO= Chloramphenicol (30 µg), CFX= Cefixime (5 µg), POL-B= Polymyxin B (300 units), IPM= Imepenem (10 ¼g), GEN= Gentamicin (10 µg), NAL= Nalidixic acid (30 µg); AZI= Azithromycin (15 µg).

Table 7: Antibiotics susceptibility pattern of the isolates from domestic waste

AntibioticsIsolates		TRI-SUL	ERY	CEF	CIP	AMP	TET	CHO	CFX	POL-B	IPM	GEN	NAL	AZI
Bulk sample	*E. coli*	S	S	ND	S	S	S	S	S	ND	R	S	R	S
	Klebsiella spp.	S	S	ND	S	R	R	S	S	ND	S	S	S	S
	Salmonella spp.	S	S	S	S	R	S	S	ND	ND	R	S	S	ND
	Pseudomonas spp.	R	ND	S	S	S	R	S	S	ND	S	S	R	S
	Bacillus spp.	R	R	v	S	S	S	S	ND	S	S	S	S	ND
	Staphylococcus spp.	S	R	S	S	S	R	S	ND	R	ND	S	ND	S
Biofilm	*E. coli*	R	S	ND	R	R	R	S	R	ND	S	S	R	R
	Klebsiella spp.	R	R	ND	R	R	R	R	S	ND	R	S	R	S
	Salmonella spp.	S	R	R	S	R	R	R	ND	ND	R	S	S	ND
	Pseudomonas spp.	R	ND	R	R	R	R	R	S	ND	S	S	R	S
	Bacillus spp.	R	R	ND	R	R	R	R	ND	R	R	S	R	ND
	Staphylococcus spp.	R	R	S	R	R	S	R	ND	R	ND	S	ND	R

ND: Not done; R: Resistant; S: Sensitive
TRI-SUL= Trimethoprime/sulfamethoxazole (25 µg); ERY= Erythromycin (15 µg); CEF= Ceftriaxone (30 µg); CIP= Ciprofloxacin (5 µg); AMP= Ampicillin (10 µg), TET= Tetracycline (30 µg), CHO= Chloramphenicol (30 µg), CFX= Cefixime (5 µg), POL-B= Polymyxin B (300 units), IPM= Imepenem (10 ¼g), GEN= Gentamicin (10 µg), NAL= Nalidixic acid (30 µg); AZI= Azithromycin (15 µg).

environment which will result in extended frequency of MDR cases and hence expand the public health risk.

Conclusion

The findings of the present study revealed that the samples from surface water, mud and different wastes contained different types of pathogenic microbes including MDR bacteria. Due to the irrational use of the surface water to serve different purposes such as bathing, washing food items, household uses etc. and the unplanned disposal of industrial, hospital and domestic wastes, these pathogenic multidrug resistance bacteria may get entry into the environment, foods or water supply systems. Due to the presence of multi-species biofilms, these may result in the drastic dissemination of MDR bacteria. Such possibilities have been justified in this study, as the multi-species biofilm formed *in vitro* from the same samples were found to enhance the antibiotic resistance. Most of the sensitive isolates were found to develop antibiotic resistance when they were isolated from biofilms. Thus the outcome of the present study vastly draws the attention for the safe disposal of industrial, hospital and municipal wastes.

Acknowledgement

We thank Microbiology Laboratory, Stamford University Bangladesh for laboratory facilities, technical assistance and financial aid.

Conflict of interest

Authors have no potential conflicts of interest.

References

1. Tenover FC. 2006. Mechanisms of antimicrobial resistance in bacteria. *Am. J. Med.,* **119**(6): S3-S10.
2. Dutta S, Hassan MR, Rahman F, Jilani MFA and Noor R. 2013. Study of antimicrobial susceptibility of clinically significant microorganims isolated from selected areas of Dhaka, Bangladesh. *Bang. J. Med. Sci.,* **12**(1): 34–42.
3. Molton JS, Tambyah PA, Ang BS, Ling ML and Fisher DA. 2013. The global spread of healthcare-associated multidrug-resistant bacteria: a perspective from Asia. *Clin. Infect. Dis.,* **56**(9): 1310–1318.
4. Khan SA, Feroz F and Noor R. 2013. Study of extended spectrum ²-lactamaseproducing bacteria from urinary tract infection in Dhaka city, Bangladesh. *Tzu Chi Med. J.,* **25**(1): 39–42.
5. Noor R, Acharjee M, Ahmed T, Das KK, Paul L, Munshi SK, Urmi NJ, Rahman F and Alam MJ. 2013. Microbiological analysis of major sea fish collected from local markets in Dhaka city, Bangladesh. *J. Microbiol. Biotechnol. Food Sci.,* **2**(4): 2420-2430.
6. Noor R, Akhter S, Rahman F, Munshi SK, Kamal SMM and Feroz F. 2013. Frequency of extensively drug resistant tuberculosis (XDR-TB) among re-treatment cases in NIDCH, Dhaka, Bangladesh. *J. Infect. Chemother.,* **19**(2): 243–248.
7. Noor R, Hossain A, Munshi SK, Rahman F and Kamal SMM. 2013. Slide drug susceptibility test for the detection of multi-drug resistant tuberculosis in Bangladesh. *J. Infect., Chemother.* **19**(5): 818-824.
8. Noor R. 2013. Identification of drug-resistant bacteria among export quality shrimp samples in Bangladesh. *Asian J. Microbiol. Biotech. Env. Sci.,* **15**(4): 31–36.
9. Aurin TH, Munshi SK, Kamal SMM, Rahman MM, Hossain S, Marma T, Rahman F and Noor R. 2014. Molecular approaches for detection of the multi-drug resistant tuberculosis (MDR-TB) in Bangladesh. *PLoS ONE,* **9**(6): e99810.
10. Mathew AG, Cissell R and Liamthong S. 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathog. Dis*. **4**(2): 115-133.
11. Munshi SK, Rahman MM and Noor R. 2012. Detection of virulence potential of diarrheagenic *Escherichia coli* isolated from surface water of rivers surrounding Dhaka City. *J. Bang. Acad. Sci.,* **36**(1): 109–121.
12. Acharjee M, Jahan F, Rahman F and Noor R. 2013. Bacterial proliferation in municipal water supplied in Mirpur locality of Dhaka city, Bangladesh. *Clean Soil Air Water,* **41**: 1–8.
13. Acharjee M, Ahmed E, Munshi SK and Noor R. 2014. Validation of *g*-irradiation in controlling microorganisms in fish. *Nutrn. Food Sci.,* **44**(3): 258-266.
14. Ahmed T, Urmi NJ, Munna MS, Das KK, Acharjee M, Rahman MM and Noor R. 2014. Assessment of microbiological proliferation and in vitro demonstration of the antimicrobial activity of the commonly available salad vegetables within Dhaka metropolis, Bangladesh. *Am. J. Agri. Forestr.,* **2**(3): 55–60.
15. Marjan S, Das KK, Munshi SK and Noor R. 2014. Drug-resistant bacterial pathogens in milk and some milk products. *Nutrn. Food Sci.,* **44**(3): 241-248
16. Scott G. 2009. Antibiotic resistance. *Med. Inter.,* **9**(4): 545-550.
17. Davison J. 1999. Genetic exchange between bacteria in the environment. *Plasmid,* **42**: 73-91.
18. Schwartz T, Kohnen W, Jansen B and Obst U. 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol. Ecolog.,* **43**: 325-335.
19. O'Toole G, Kaplan HB and Kolter R. 2000. Biofilm formation as microbial development. *Annu. Rev. Microbiol*., **54**: 49–79.
20. Donlan RM and Costerton JW. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.,* **15**: 167–193.
21. Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ and Kjelleberg S. 2006. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl. Environ. Microbiol., **72**(6): 3916–3923.
22. Adal KA and Farr BM. 1996. Central venous catheter-related infections: a review. *Nutrition,* **12**(3): 208–13.
23. Archibald LK and Gaynes RP. 1997. Hospital acquired infections in the United States: the importance of interhospital comparisons. *Nosocom. Infect*., **11**(2): 245–55
24. Kalmbach S, Manz W and Szewzyk U. 1997. Dynamics of biofillm formation in drinking water: phylogenetic aD liation and metabolic potential of single cells assessed by formazan reduction and in situ hybridization. *FEMS Microb. Ecol*., **22**: 265-279.
25. Schwartz T, Ho¡mann S and Obst U. 1998. Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. *Water Res*., **32**: 2787-2797.
26. Cappuccino JG and Sherman N. 1996. Microbiology - A laboratory manual. 4th ed. The Benjamin/Cummings Publishing Co., Inc., Menlo Park, California.
27. Colwell RR. 2000. Non-culturable microorganisms in the environment. American Society of Microbiology, Washington DC, USA.
28. Oliver JD. 2005. The viable but nonculturable state in bacteria. *J. Microbiol.,* 43, 93-100.
29. Rahman F. and Noor R. 2012. Prevalence of pathogenic bacteria in common salad vegetables of Dhaka metropilis. *Bang. J. Bot.,* **41**(2): 159-162.
30. Alfrad EB. 2007. Bensons microbiological applications. Mcgraw-Hill Book Company, New York.

31. Baum MM, Kainovic A, O'Keeffe T, Pandita R, McDonald K, Wu S and Webster P. 2009. Characterization of structures in biofilms formed by a *Pseudomonas fluorescens* isolated from soil. *BMC Microbiol*., **9**(1): 103.

32. Bauer AW, Kirby WMM, Sherris JC and Tierch M. 1968. Antibiotic susceptibility testing by a standardized single disc method. *American J. Clin. Pathol,* **45**(4): 493-496.

33. Ferraro MJ, Craig WA and Dudley MN. 2001. Performance standards for antimicrobial susceptibility testing. NCCLS, Pennsylvania, USA.

34. Wang H, Dzink-Fox JL, Chen M and Levy SB. 2001. Genetic characterization of highly fluoroquinolone-resistant clinical *Escherichia coli* strains from China: role of acrR mutations. *Antimicrob. Agents Chemother.,* **45**: 1515–1521.

35. Woodford N and Ellington MJ. 2007. The emergence of antibiotic resistance by mutation. *Clin. Microbiol. Infect*., **13**: 5–18.

36. Ochman H, Lawrence JG and Groisman EA. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature,* **405**: 299- 304S.

37. Davies J and Davies D. 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.,* **74**: 417-433.

38. Lee HH, Molla MN, Cantor CR and Collins JJ. 2010. Bacterial charity work leads to population-wide resistance. *Nature,* **467**: 82-86.

39. Choudhury R, Panda S and Singh DV. 2012. Emergence and dissemination of antibiotic resistance: A global problem. *Indian J. Med. Microbiol.,* **30**(4): 384-390.

40. Acharjee M, Rahman F, Beauty SA, Feroz F, Rahman MN and Noor R. 2011. Microbiological study on supply water and treated water in Dhaka city. *S. J. Microbial*., **1**(1): 42-45.

41. Kolwzan B, Traczewska T and Pawlaczyk-Szipilowa M. 1991. Preliminary examination of resistance of bacteria isolated from drinking water to antibacterial agents. *Environ. Prot. Eng.,* **17**: 53-60.

42. Korber DR, Choi A, Wolfaardt GM, Ingham SC and Caldwell DE. 1997. Substratum topography influences susceptibility of *Salmonella enteritidis* biofilms to trisodium phosphate. *Appl. Environ. Microbiol.,* **63**: 3352-3358.

43. Chowdhury FFK, Acharjee M and Noor R. 2016. Maintenance of environmental sustainability through microbiological study of pharmaceutical solid wastes. *Clean Soil Air Water,* **44**(3): 309-316.

44. Cloete TE. 2003. Resistance mechanisms of bacteria to antimicrobial compounds. *Int. Biodetern. Biodegradn*., **51**(4): 277-282.