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Role of heavy metals on antibiotic resistance properties in lactose fermenting isolates collected from textile industry effluents

Rokunozzaman, Afroza Parvin, Md. Mahmudul Hasan, Papon Debnath, Md. Ashraful Hasan, Md. Mozammel Hossain, Mohammad Mahfuz Ali Khan Shawan, Abdul Halim, Mohammad Azizur Rahman, Sabir Hossain and Sohel Ahmed*

Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

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It is crucial to monitor the microbial ambience in heavy metal enriched industrial effluents that generally discharge into the environment. Therefore, the present study aimed to understand the load of heavy metals in multidrug-resistant lactose fermenting microorganisms isolating from textile industry effluents. Samples were collected from five different textile industrial canals. In most of the cases, the colony forming units (CFUs) per plate were uncountable at 10^{-6} dilution but countable at 10^{-10} dilution. A total of 100 lactose fermenting (LF) isolates were selected using 4 differential media and tested for the determination of minimum inhibitory concentrations (MICs) of heavy metals and antibiotic susceptibility. For nickel (Ni), the MIC was 0.3 mM for almost 98% isolates. The MIC was 0.5 mM for chromium (Cr) in almost 99% isolates. For lead (Pb), 100% isolates had a MIC of 0.4 mM. The most prevalent (36%) resistance pattern was found for sulfamethoxazole-trimethoprim (SXT) and only 10% isolates showed resistance to cefotaxime (CTX). The combined effect of heavy metals and antibiotics revealed that in most cases, the antibiotic zone of inhibition was increased. Plasmid profiling showed that among 14 selective isolates, high sized (21kb) plasmid was found in 6 isolates.

Key words: Antibiotics, effluents, heavy metals, minimum inhibitory concentration (MIC), antimicrobial resistance.

INTRODUCTION

Wastewater is widely known as crucial crossroads between diverse water bodies, for example, wastewater from hospital, household water, surface- and ground-water (Zhang et al., 2009). However, this study is mainly focusing on industrial wastewater discharged directly to the environment as effluents during the pre- and post-processing of textile products. The wastewater is actually outlined by the presence of discriminating pressures of harmful chemical compounds, heavy metals (copper, lead, chromium, nickel, cadmium, zinc etc.), and high

*Corresponding author. Email: drsahmed_bmbju@juniv.edu Tel: +8801706585499.

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content of organic materials (Kümmerer, 2003; Martinez et al., 2009; Rahube and Yost, 2010) which contribute an opportunity for the mobile elements to be incorporated into the opportunistic pathogenic microbes and naturally growing environmental bacteria (Martinez et al., 2009; Sørensen et al., 2005; Szczepanowski et al., 2009). Furthermore, once the genes responsible for antibiotic resistance are successfully integrated in gene-transmission components, they can be persistent and spread between discrete microbial species alike in the absence of antibiotic compounds (Abd Elhady et al., 2020; Allen et al., 2010; Khan, 2016; Koike et al., 2007; Martinez et al., 2009). In Bangladesh, very little information is acknowledged regarding the natural- and human-associated environmental repositories of resistance in polluted or contaminated water from textile industries. Moreover, most of the textile industries do not have the effective effluent treatment plants (ETP), although there are strict rules and regulations imposed by the government.

Urban wastewater serves as important repositories of human and animal symbiotic bacterial species in which antimicrobial resistance determinants and/or micro-organisms remain in the eventual effluents and are dispensed and/or released into the environment without any significant pre-treatments (Reinthaler et al., 2003; Tennstedt et al., 2003, 2005). In addition, by conducting a comparative study on three activated sludge-treatment plants, it was concluded that although there were no significantly noticeable increases in antibiotic resistance phenotypes detected over the period of sewage treatments, these processes may contribute to the circulation of resistant bacteria mainly to the water and soil environment (Reinthaler et al., 2003; Tennstedt et al., 2003, 2005). Additionally, Tennstedt et al. (2003) described the existence of antibiotic resistance markers in self-transmissible genetic elements of bacteria inhabiting the activated-sludge and effluent end products released from a wastewater treatment plant. In this study, the prevalence or the predominance of antibiotic and heavy metal resistance was investigated in lactose-fermenting (LF) bacterial isolates collected from wastewater containing textile effluents.

Bangladesh has a significant number of textile, dying and tannery industries, however, till now there were no systemic and extensive studies in this area except some random and small scale studies; for instance, Ashikuzzaman et al. (2015) studied gram positive bacteria with heavy metal resistance isolated from textile effluents. In addition, Md et al. (2009) studied on chromium resistance bacteria and Hossain and Anwar (2012) reported on chromium and copper resistance bacteria isolated from tannery effluents. As a result, the present study was a sure systemic and a small-scale effort to study on gram negative lactose fermenting *Enterobacteriaceae* providing emphasis on mostly phenotypic characterization.

MATERIALS AND METHODS

Study area and sample collection

The samples from textile effluents were collected in sterilized airtight 1.5-L glass bottles (Pyrex, UK) from the canals of Savar Upazila within three months of the period from June to August in 2016. The effluents discharged directly from different textile industries into the canals and the canals are connected to the nearby Bangsi and Turag rivers. Effluent samples were collected from five (n = 5) different canals and from each canal, five samples were collected with average distance of 2 m. The collected samples were transported to the laboratory and stored at 4ºC for further studies. The name of the industrious canals, dates of collection, and distances of the site of collection were given in the Table 1 and also the map of Savar Upazila was shown in Figure 1, where locations of all the textile industries were indicated including the nearby rivers. The effluents are generally discharged from different textiles through the canals. The red color indicated the different canal sites from where the samples were collected. The present study was carried out in the Common Research Laboratory as well as in the Research Laboratory for Biomedical Sciences, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka.

Total bacterial colony (TBC) count

The textile effluent samples were serially diluted with autoclaved distilled water from 10^{-1} to 10^{-10}. Plate counting agar (PCA) media was used to count the total bacterial colony by spreading the serially diluted effluents 10^{-6} to 10^{-10}, that is, serially, total of 5 PCA plates were used for each sample of each location. Each plate (20 ml media) was inoculated with 50 µl aliquot (2.5 µl/ml) and spread with the spreader followed by incubation at 37ºC for 5 consecutive days to get the total bacterial colony (TBC). The appeared colony forming units (CFUs) were counted manually observing under light microscope (XSZ-107 BN 230 v, USA).

Selection of gram-negative LF colonies using differential media

To enrich the number of bacteria, 1 ml aliquot of the collected effluent from each sample was inoculated in 1 ml of autoclaved nutrient broth (NB) and incubated for 24 h in an orbital shaker incubator at a shaking speed of 180 rpm at 37°C. After incubation, the culture was inoculated in agar plates containing differential media using cotton swab and by streaking. Four differential media, such as MacConkey (MAC) agar, Xylose-Lysine-Deoxycholate (XLD) agar, *Shigella-Salmonella* (SS) specific agar and Thiosulphate-Citrate-Bile Salt-Sucrose (TCBS) agar were used to identify gram negative lactose fermenting *Enterobacteriaceae* colonies (Bacteriological Analytical Manual, 1998). The different visible colonies on differential agar plates were selected, picked, cultured and purified by repeated plate streaking method (Cappuccino et al., 2017). The purity of each bacterial colony morphology of the selected lactose fermenting *Enterobacteriaceae* isolates was ensured by observing their color, size and shape by comparing with those of the standard colonies of *Escherichia coli* (*E. coli*; ATCC 25922) (UniProt accession number: CP009072) under the microscope. These standard colonies that grew on the four differential media were used as positive control. The non-lactose fermenting (NLF) colonies were also isolated following the

Table 1. Information of specific locations in canals carrying textile industry effluent.

Sample	Date of collection	Location	Distance	Industrial canal
S1	June 26, 2016	L1----L2	2.0 m	Pakiza Textiles Ltd.
		L2----L3	1.5 m	
		L3----L4	2.5 m	
		L4----L5	2.0 m	
S2	August 1, 2016	L1----L2	1.0 m	Aman Spinning Mills
		L2----L3	2.0 m	Mondal Fashions Ltd.
		L3----L4	1.5 m	Green Life Knit Composite Ltd.
		L4----L5	3.0 m	Eva Garments
S3	August 7, 2016	L1----L2	2.5 m	Marma Composites
		L2----L3	2.0 m	
		L3----L4	1.5 m	Fashion Garments Ltd.
		L4----L5	2.0 m	
S4	August 21, 2016	L1----L2	1.0 m	Ultra Embroidary Ltd.
		L2----L3	3.0 m	
		L3----L4	1.5 m	Amigo Fashion Ltd.
		L4----L5	2.5 m	Shine Embroidary Ltd.
S5	August 28, 2016	L1----L2	1.0 m	Dekko Designs Ltd.
		L2----L3	3.0 m	Magpie Knit Wear
		L3----L4	1.5 m	Hameem Group AJ Super Garments Ltd.
		L4----L5	2.5 m	Sharmin Group Trouser Line Ltd.

S: Sample; L: Location; M: Meter.

same procedure where *Shigella flexneri* 2a (UniProt accession number: AE005674), and *Salmonella* Serovar paratyphi B (UniProt accession number: CP000886) standard colonies were used. All the selected isolates were cultured and grown in NB media supplemented with 0.3% yeast extract and stored at -80°C temperature after adding 15% glycerol.

Determination of minimum inhibitory concentrations (MICs) to heavy metals

The MICs of different toxic heavy metals were determined by preparing MAC agar supplementing with three metal salts, such as $K_2Cr_2O_7$ (chromium salt), $NiSO_4$ (nickel salt) and $PbNO_3$ (lead salt) to give a final concentration of 0.1, 0.3, 0.5, 1.0, and 2.0 mM for Cr^{6+}, 0.2, 0.3, 0.6, 1.25, 2.5 mM for Ni^{2+} and 0.1, 0.15, 0.3, 0.6, 1.2 mM for Pb^{2+}, respectively. Spot inoculations were performed using plate assay method by drawing square patches on the plates. The plates were marked with 40 patches. Next, each patch of the plates was inoculated with a single colony taken from previously cultured MAC agar and incubated at 37°C for 24 h (Zhou et al., 2015). As a negative control, *E. coli* (ATCC 25922) was used.

Antibiotic susceptibility test

On Mueller-Hinton (MH) agar, the isolates were screened on modified Kirby-Bauer disc diffusion method using the standard guidelines (CLSI, 2016; Talukder et al., 2002). The tested antibiotics were cefotaxime (CTX, 30 µg), sulfamethoxazole-trimethoprim (SXT, 25 µg), tetracycline (TE, 30 µg), azithromycin (AZM, 15 µg) and ciprofloxacin (CIP, 5 µg) (Bio-Rad, USA). *E. coli* ATCC (25922) was used as a negative control.

Binary exposure experiment

Individual MH agar plates supplemented with individual heavy metal salts (nickel/chromium/lead) were prepared based on the results of MIC and antibiotic susceptibility tests. For nickel, chromium, and lead, single MH agar plates with 0.6 mM $NiSO_4$, 1.0 mM $K_2Cr_2O_7$, and 0.6 mM $PbNO_3$ were prepared, respectively. After adding individual metal salts to MH agar plates, each individual bacterial colony was then spread on the plates and allowed to grow for 10 min. Then antibiotic discs containing 30 µg cefotaxime (CTX), 25 µg sulfamethoxazole-trimethoprim (SXT), 30 µg tetracycline (TE), 15 µg azithromycin (AZM) and 5 µg ciprofloxacin (CIP) (Bio-Rad, USA) were placed on the inoculated plates following standard CLSI guidelines as described (CLSI, 2016; Talukder et al., 2002) and incubated for a period of 24 h at 37°C. Based on the zone of inhibition values measured in millimeters, the effects of metal salts on the spectrum of antimicrobial resistance were analysed (Zhou et al., 2015). A negative control [*E. coli* ATCC (25922)] was used and experimental results were repeated in triplicates (n=3) to verify their

Figure 1. Map showing the different textile industries of Savar Upazila (red color is pointing the different canal sites of different textile industries).

reproducibility.

Plasmid profiling

The plasmid DNA was extracted using the simplified alkaline lysis method developed by Kado and Liu (1981) with minor modifications, followed by electrophoresis using 0.8% agarose in Tris-Borate EDTA buffer (Talukder et al., 2002; Kado and Liu, 1981). In this experiment, reference strain *E. coli* ATCC (25922) does not carry any plasmid and was therefore used as a negative control. Plasmid bearing *E. coli*, R1 (62 MDa) and V517 (35,6,4,7,3,3,2,1 I,1.8,1.4 MDa) were taken as positive controls (Talukder et al., 2002; Henry, 1991). Molecular weights of plasmids found between the reference strains were converted to Kilo base pair (Kb) measurements. Molecular weight of the plasmids was determined by comparing them to the circular DNA ladder molecular weight markers of 80-10,000 bp (Mass Ruler DNA

Table 2. Day-wise breakup of total bacterial colony (TBC) counts at varying dilution.

Sample ID	Plate no.	Dilution factor	Total bacterial colony (TBC) count by plate observation				
			Day 1	Day 2	Day 3	Day 4	Day 5
			(CFUs/plate)				
S1L1	1	10^{-6}	42	45	48	N	N
	2	10^{-7}	36	39	43	N	N
	3	10^{-8}	24	27	33	46	N
	4	10^{-9}	12	18	21	38	N
	5	10^{-10}	7	8	15	23	N
S1L5	1	10^{-6}	N	N	N	N	N
	2	10^{-7}	N	N	N	N	N
	3	10^{-8}	2	5	8	N	N
	4	10^{-9}	2	3	5	N	N
	5	10^{-10}	1	2	4	9	N
S2L1	1	10^{-6}	69	N	N	N	N
	2	10^{-7}	40	N	N	N	N
	3	10^{-8}	23	23	30	N	N
	4	10^{-9}	10	13	13	16	18
	5	10^{-10}	4	8	10	14	17
S2L5	1	10^{-6}	N	N	N	N	N
	2	10^{-7}	15	21	27	38	53
	3	10^{-8}	9	16	16	18	41
	4	10^{-9}	8	13	14	21	35
	5	10^{-10}	6	18	20	23	27
S3L1	1	10^{-6}	N	N	N	N	N
	2	10^{-7}	N	N	N	N	N
	3	10^{-8}	N	N	N	N	N
	4	10^{-9}	11	14	16	19	N
	5	10^{-10}	7	9	16	19	23
S3L5	1	10^{-6}	53	57	61	77	83
	2	10^{-7}	45	48	50	56	63
	3	10^{-8}	31	37	44	49	51
	4	10^{-9}	23	26	31	37	41
	5	10^{-10}	13	19	23	28	32
S4L1	1	10^{-6}	N	N	N	N	N
	2	10^{-7}	N	N	N	N	N
	3	10^{-8}	N	N	N	N	N
	4	10^{-9}	51	59	63	74	N
	5	10^{-10}	27	33	48	52	N
S4L5	1	10^{-6}	N	N	N	N	N
	2	10^{-7}	47	51	60	66	71
	3	10^{-8}	39	45	55	57	63
	4	10^{-9}	24	31	43	49	56
	5	10^{-10}	11	16	21	26	35
S5L1	1	10^{-6}	N	N	N	N	N
	2	10^{-7}	N	N	N	N	N
	3	10^{-8}	12	33	37	41	N
	4	10^{-9}	11	29	31	35	45
	5	10^{-10}	7	23	28	32	37
S5L5	1	10^{-6}	20	42	N	N	N
	2	10^{-7}	11	29	N	N	N

Table 2. Contd.

3	10^{-8}	9	14	17	19	24
4	10^{-9}	8	13	15	20	22
5	10^{-10}	2	4	5	8	15

SL: Sample location; N: Numerous; ID: Identification; CFU: Colony forming unit.

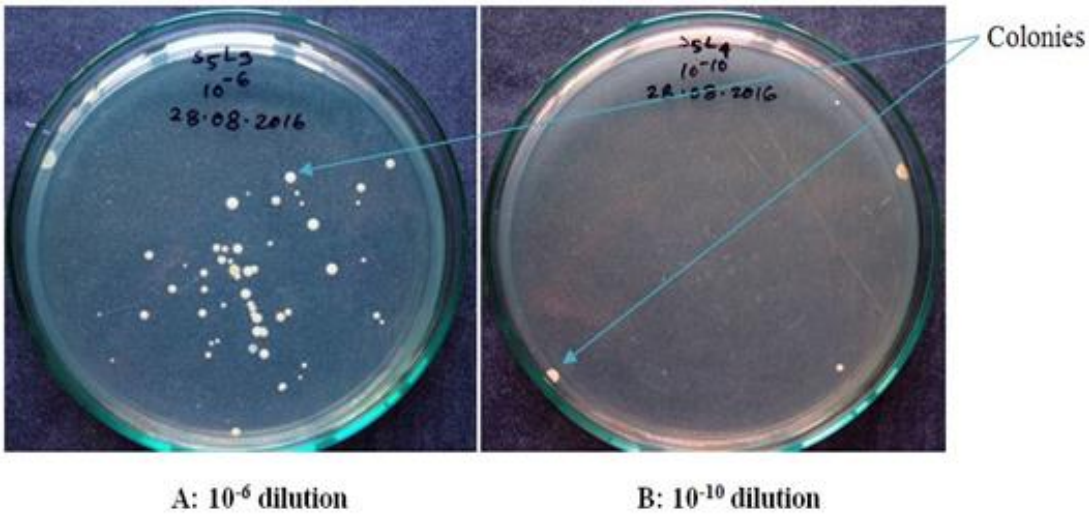

Figure 2. Representative plate observing and counting the CFUs manually in day 1.

Ladder Mix, Thermo Scientific, USA). The plasmid extraction protocol as well as the size of the plasmids were validated through the use of these negative and positive control strains.

Statistical analysis

Analysis of the data was conducted using MS-Excel, R 3.5.3 and RStudio for Windows 10 (32/64 bits). In order to analyze the data, the linear model was used to perform an ANOVA with one tail t-test and a p-value of 0.05 was considered statistically significant. The binary exposure experiment results were expressed as the means and standard deviations of three replications.

RESULTS AND DISCUSSION

Total bacterial colony (TBC) count found in textile samples

The number of colony forming units (CFUs) for each sample of each dilution for a single day and for two locations (1 and 5) was given in Table 2 and Figure 2 shows the plate with colonies which was grown in PCA media. Sample 3 was collected from the canals coming from Marma Composites and Fashion Garments Ltd. In day 1, for 10^{-6} dilution, N number CFUs/plate was observed. On the other hand, for 10^{-10} dilution, in day 1, average 10 CFUs/plate was found. On day 5, for 10^{-6} dilution, average N number CFUs/plate was observed and for 10^{-10} dilution, average N number CFUs/plate was counted also. For other samples, the total observations were given in Table 2. The results were almost consistent for the other samples as well (Table 2). The level of CFUs/plate definitely exceeded the allowable standard CFU per 2.5 µl volume of effluents. For *E. coli,* the plate count was linear and ranged from 30 to 300 CFU on a standard or regular sized petri-dish. Therefore, to ensure that a sample will yield CFU in this range requires dilution of the sample and plating of several dilutions. Generally, for this experiment, ten-fold microbial dilutions were used (Breed and Dotterrer, 1916).

Selection of gram-negative LF colonies

By microscopic and naked eye observation, 20 gram-negative LF colonies were isolated (Figure 3) using four differential media from five locations of each canal (average 4 colonies from each location). By comparing with a positive control *E. coli* ATCC (25922), well-defined, round, concave shaped colonies found in MAC (pink coloured colonies) or SS (faint pink coloured colonies), XLD (yellow coloured colonies) agar and TCBS (small

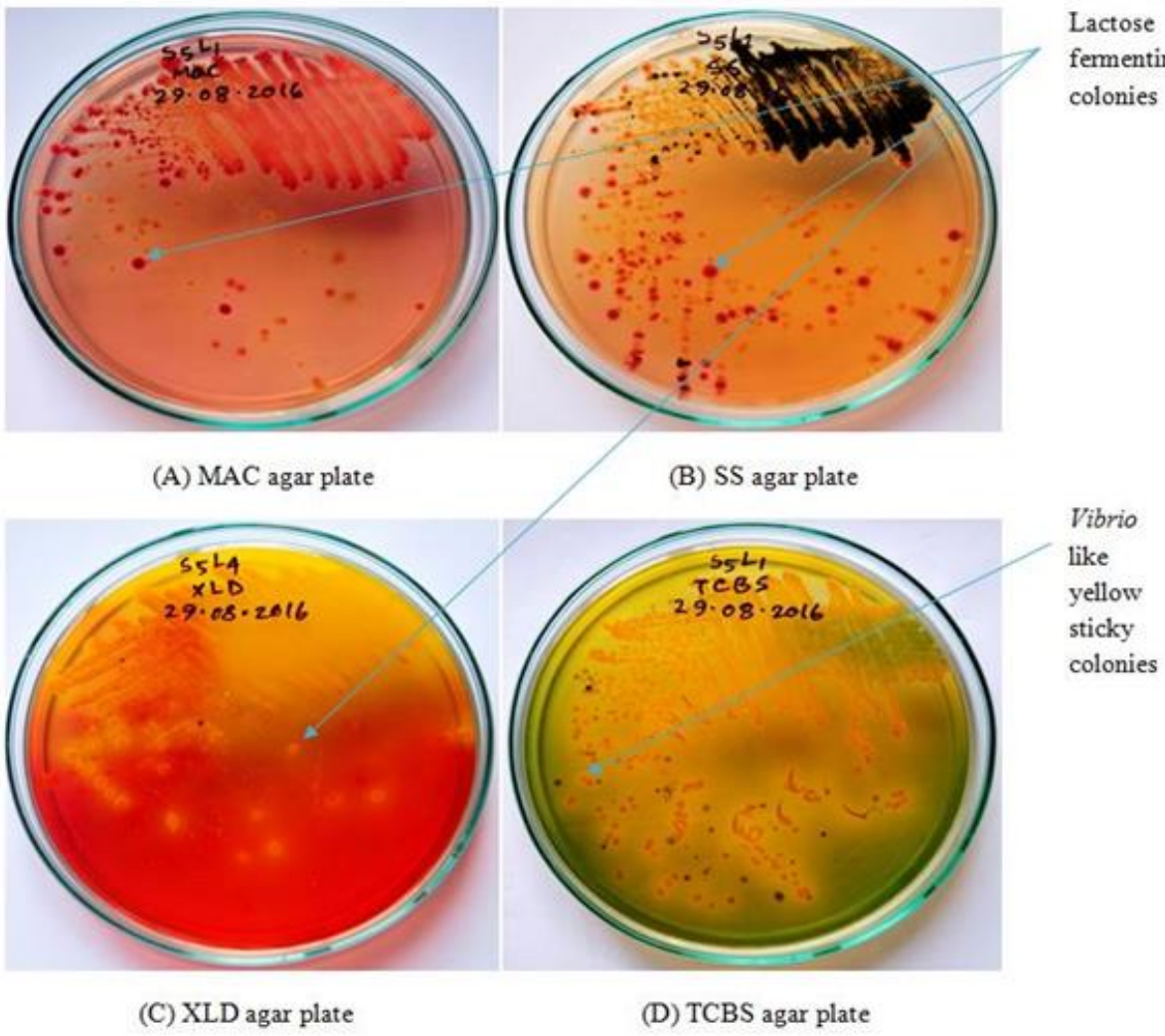

Figure 3. Representative photographs showing colony morphologies of LF and Vibrio like colonies.

clear colonies) were isolated as LF colonies.

A total of 100 LF isolates were selected from five different samples each with five different locations and stored at -4°C for further investigation. In this study, 100 NLF isolates were also isolated and stored; however, only LF isolates were characterized in this study. In general, the colonies formed in culture by different species of bacteria are quite different and are clearly distinguishable by both micro- and macroscopic observations. For observations and identification of growing available microorganisms on plates, the morphology of colonies could be of great use (Practical Handbook of Microbiology, 2015).

A normal Q-Q plot was observed in a linear model for 100 NLF and 100 LF isolates (Figure 4). In ANOVA analysis, a highly significant *p*-value (< 0.05) and a small t-scored value (0.120863) was found indicating significant similarity exists between the two sets of samples (NLF and LF isolates).

Determination of MICs to heavy metals of representative LF isolates

MICs (Figure 5) of the 100 LF bacterial isolates was tested for their resistance to 3 different metals where almost all the isolates displayed resistance to these metals tested (Ni, Cr and Pb). This is because the most common heavy metal pollutants in the environment are Cr, Mn, Ni, Cu, Zn, Cd, and Pb (Fu et al., 2017). In this experiment, 98% (n=98) isolates were grown in 0.2 mM and 0.3 mM concentrations of Ni (nickel) whereas none (n=0) of the isolates was grown in 1.25 mM and 2.5 mM concentrations of Ni. Only 3% (n=3) isolates were able to grow in 0.6 mM concentration of Ni. For Cr (chromium), 100% (n=100) isolates were able to grow in 0.1 mM and 0.3 mM concentrations. Ninety-nine percent (n=99) and 95% (n=95) isolates were able to tolerate the 0.5 mM and 1.0 mM concentrations of Cr, respectively. Only 17% (n=17) isolates had a maximum

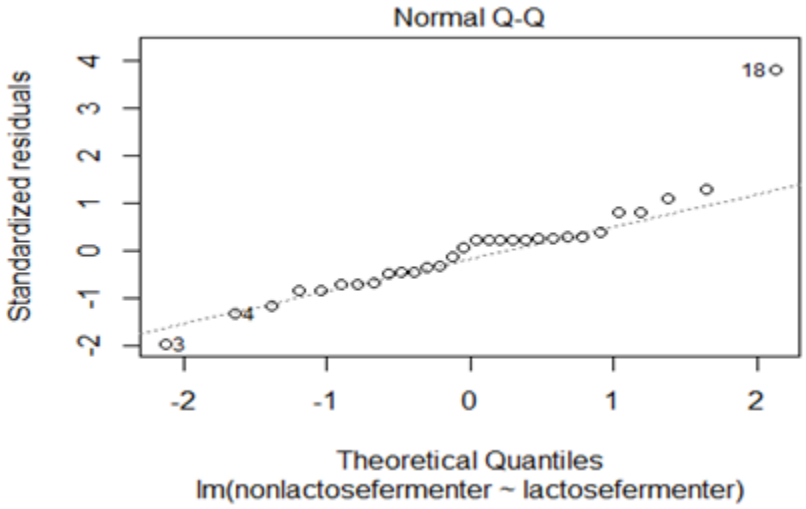

Figure 4. A linear model indicating a normal Q-Q plot for two sets of samples (NLF and LF isolates).

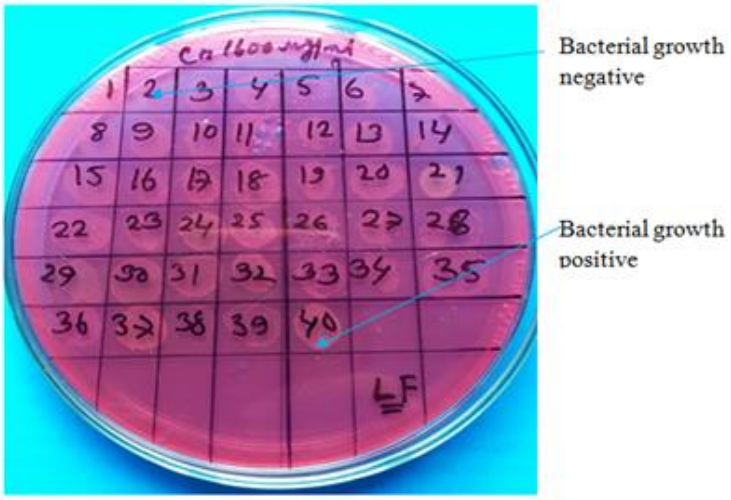

Figure 5. Representative photographs of LF colonies showing MICs of heavy metals determined by plate assay method.

tolerable concentration of 2.0 mM Cr. For Pb (lead), 100% (n=100) isolates were tolerable to 0.1, 0.2 and even 0.4 mM concentrations and 99% (n=99) isolates were also able to grow in 0.6 mM concentration. Only 20% (n=20) isolates had a maximum tolerable concentration 1.2 mM Pb. In Table 3, MICs for representative LF isolates (n=23) and *E. coli* ATCC (25922) as a negative control were given. Table 4 provides overall information about percentage (%) of the LF isolates able to tolerate different concentrations of three different heavy metals. It is also important to note that 17% (n=17) isolates were tolerable to 2.0 mM Cr concentration and 20% (n=20) isolates were tolerable to 1.2 mM Pb concentration from isolated from sample 3 (Marma Composites and Fashion Garments Ltd.). The capability of showing resistance by these bacterial isolates could be as a result of their surviving in the textile effluents containing high concentrations of all types of heavy metals.

One study (Wright et al., 2006) reported that bacteria samples including a gradient of contaminated heavy metals displayed greater tolerance to those metals as well as antibiotics compared to bacterial samples collected from a standard reference site. The experimental findings of our present study revealed the high heavy metal resistance in bacterial isolates from textile industrial effluents. This result is supported by another experiment conducted by Aleem et al. (2003)

Table 3. MICs of representative LF isolates (n=23) to heavy metals.

Lab ID	Heavy metals														
	Nickel (Ni) Conc. (mM)					Chromium (Cr) Conc. (mM)					Lead (Pb) Conc. (mM)				
	0.2	0.3	0.6	1.25	2.5	0.1	0.3	0.5	1.0	2.0	0.1	02	0.4	0.6	1.2
1	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
5	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
15	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
23	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-
25	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
27	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
29	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
31	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
34	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
35	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
39	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
44	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
46	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
49	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
51	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
59	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
60	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+
68	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
73	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
83	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
11	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
85	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
99	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-
ATCC (25922)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ID: Identification; Conc.: Concentration; mM: Milimolar, ATCC (25922): *E. coli* negative control strain; “+”: Growth positive; “-”: Growth negative.

Table 4. MICs (%) of representative LF isolates (n=100) to heavy metals.

Isolate	Conc. of Ni (mM)				
	0.2	**0.3**	**0.6**	**1.25**	**2.5**
Lactose fermenter	98%	98%	3%	0	0
	Conc. of Cr (mM)				
	0.1	**0.3**	**0.5**	**1.0**	**2.0**
Lactose fermenter	100%	100%	99%	95%	17%
	Conc. of Pb (mM)				
	0.1	**0.2**	**0.4**	**0.6**	**1.2**
Lactose fermenter	100%	100%	100%	99%	20%

ID: Identification; Conc.: Concentration; mM: Milimolar; Ni: Nickel; Cr: Chromium; Pb: Lead.

who showed that 77·10% of the bacterial isolates collected from the agricultural soil of Aligarh and treated with the samples of wastewater exhibited resistance to most of the toxic heavy metals up to 71·4, 65.7, 65.7, 54.2, 45.7 and 25.7% for Cr, Cd, Zn, Cu and Hg respectively. In another study, Murtaza et al. (2002) illustrated that of 80 *E. coli* isolates cultured and collected from five different regions of India, 4 regions were

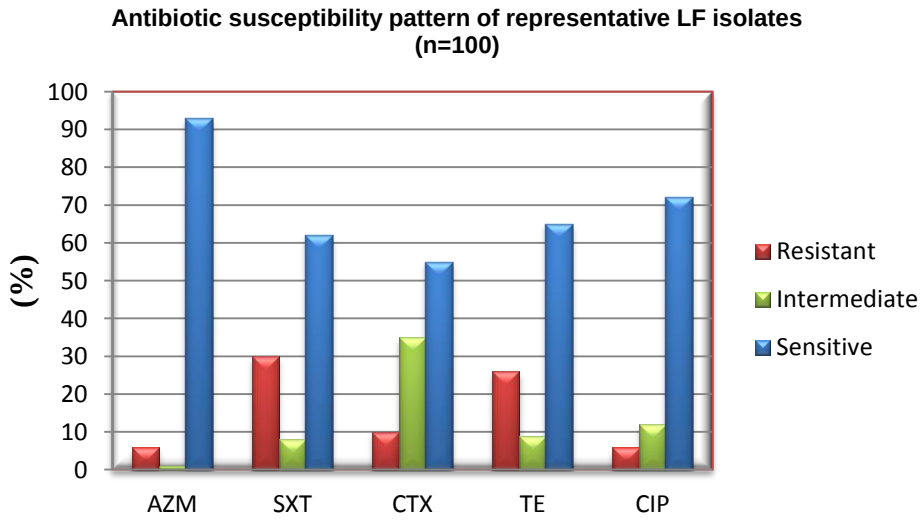

Figure 6. Distribution (%) of antibiotic susceptibility pattern of representative LF isolates (n=100) (CIP: Ciprofloxacin; CTX: Cefotaxime; AZM: Azithromycin; SXT: Sulfamethoxazole-trimethoprim; TE: Tetracycline).

contaminated with effluents from household and industry while 1 was from unpolluted area where 68 isolates displayed resistance to at least any one of these metals-Cu, Cd, Pb, Co, Zn, and Fe. The bacterial resistance to heavy metals possibly indicates the intensity of environmental contamination by these toxic metals and might be directly linked to the bacterial routine exposure to those metals. On the other hand, there is a possibility of harbouring heavy metal resistant microorganisms on the uncontaminated environment or organisms may be spontaneously adapted to high toxic metal concentrations (Murtaza et al., 2002).

Determination of antimicrobial susceptibility test of representative LF isolates (n=100)

In this experiment, five antibiotics were chosen on the basis of their importance in treating human or animal *Enterobacteriaceae* infections and their use as feed additives to promote growth in animals and also on the basis of their ability to provide diversity for representation of different antimicrobial agent classes (Butler and Paterson, 2020). Among 100 LF isolates, the most prevalent (36%, n=36) resistance pattern was found for sulfamethoxazole-trimethoprim (SXT) followed by 26% (n=26) for tetracycline (TE). For azithromycin (AZM) and ciprofloxacin (CIP), the percentages for resistance pattern were equal, estimating 6% (n=6) for each which was also the lowest resistance pattern. For cefotaxime (CTX), 10% (n=10) isolates were found to be resistant to this antibiotic.

It is interesting to note that 35% (n=35) isolates were showing intermediary resistance pattern to CTX whereas only 1% (n=1) for AZM. For CIP, TE and SXT, the intermediary resistance pattern was found 12% (n=12), 9% (n=9) and 8% (n=8), respectively. Overall, 16% (n=16) isolates were found to be multidrug (more than one drug) resistant and another 12% (n=12) isolates were observed to be intermediary resistant (Figure 6). Therefore, it is possible that all these intermediary resistant isolates would turn into complete resistant gradually within a short duration of time increasing the number of multidrug-resistant isolates. Isolation of antibiotic resistant *Enterobacteriaceae* showed the contamination of industrial effluents with nearby sewage waste water. The strains of bacteria isolated from the soil of agricultural land exposing continuously by contaminated waste water exhibited high heavy metal and antibiotic resistance (Wright et al., 2006).

Bacterial isolates from the contaminated soils (Máthé et al., 2012), surface water (Koc et al., 2013) and even the shallow sediments of Antarctica (Lo Giudice et al., 2013), have also been reported to show cross-resistance to toxic heavy metal and antibiotic. The findings of the current study agreed with the findings of the previous studies. The water and soil of industrial environments are regularly contaminated with a variety of organic and inorganic pollutants (Aleem et al., 2003; Aleem and Malik, 2003; Ansari and Malik, 2007). In these environmental samples, multi-resistant bacteria are clearly correlated to the resistance or tolerance of the hazardous heavy metals. The heavy metals and various drugs including antibiotics are environmentally exposed factors resultant from pollution caused by human which employ a selective pressure for the development of resistance in bacteria (Lazăr et al., 2002). The detailed mechanisms of cross-resistance to both heavy metals and antibiotics in

Table 5. Antibiotic susceptibility pattern for representative LF isolates (n=23).

Lab ID	Isolate ID	Antibiotic Discs					Resistance to antibiotics
		CIP (5 µg)	CTX (30 ug)	AZM (15 ug)	SXT (25 µg)	TE (30 µg)	
1	S1L1 LF1	S	R	S	R	S	CTX, SXT
5	S1L2 LF1	S	R	S	R	S	CTX, SXT
15	S1L4 LF3	S	R	S	R	S	CTX, SXT
23	S2L1 LF3	R	R	I	R	I	CIP, CTX, SXT
25	S2L2 LF1	S	R	I	R	I	CTX, SXT
27	S2L2 LF3	R	R	I	R	S	CIP, CTX, SXT
29	S2L3 LF1	S	R	R	R	R	CTX, AZM, SXT, TE
31	S2L3 LF3	S	R	R	R	R	CTX, AZM, SXT, TE
34	S2L4 LF2	S	R	I	S	S	CTX
35	S2L4 LF3	S	R	I	S	S	CTX
39	S2L5 LF3	R	R	S	R	I	CIP, CTX, SXT
44	S3L1 LF4	S	I	R	S	I	AZM
46	S3L2 LF2	S	R	R	S	I	CTX, AZM
49	S3L3 LF1	S	R	S	R	R	CTX, SXT, TE
51	S3L3 LF3	S	R	I	R	R	CTX, SXT, TE
59	S3L5 LF3	S	R	R	R	R	CTX, AZM, SXT, TE
60	S3L5 LF4	S	S	I	R	R	SXT, TE
68	S4L2 LF4	S	S	I	R	S	SXT
73	S4L4 LF1	S	S	R	S	S	AZM
83	S5L1 LF3	S	I	R	I	S	AZM
11	S1L3 LF3	S	S	S	S	S	-
85	SFL2 LF1	S	S	S	S	S	-
99	S5L5 LF3	S	S	S	S	S	-
ATCC	25922	S	S	S	S	S	-

ID: Identification; LF: Lactose fermenter; CIP: Ciprofloxacin; CTX: Cefotaxime; AZM: Azithromycin; SXT: Sulfamethoxazole-trimethoprim; TE: Tetracycline; S: Sensitive; I: Intermediary; R: Resistance.

bacterial isolates are still unclear.

The number of resistant isolates obtained from sample 2 (6%, n=6) and sample 3 (4%, n=4) were more than that of samples 1, 4 and 5. Even 3% (n=2) and 2% (n=2) isolates were intermediary resistant which were isolated from sample 2 and 3, respectively. In Table 5, significant antibiotic susceptibility patterns for 23 LF isolates were given. From this finding it is assumable that perhaps samples 2 and 3 were more contaminated with different metals as contamination of metals directly selects metal-tolerant bacteria and at the same time co-selects antibiotic-tolerant bacteria (Wright et al., 2006).

Binary exposure experiment of representative LF isolates (n=14)

By binary exposure experiment, the effects of heavy metals on diameter of zone of inhibition of isolates to antibiotics were observed. Based on the results of MICs and antimicrobial susceptibility tests, ten isolates were finally selected for this experiment. The isolates, which were resistant to at least one antibiotic and had intermediary resistance for at least one antibiotic, were selected. Three types of MH agar plates were prepared adding Ni (0.3 mM), Cr (1.0 mM) and Pb (0.6 mM) separately. The specific metal concentrations were selected based on the maximum isolates tolerable to that specific concentration. Among 10 isolates, 9 isolates were able to tolerate these specific concentrations. Three more isolates were also taken which were susceptible to all antibiotics but still able to tolerate these specific metal concentrations. *E. coli* ATCC (25922) was included as a negative control. In Table 6, a zone of inhibition of antibiotics was given in the presence of Ni (0.3 mM), Cr (1.0 mM), Pb (0.6 mM) of representative 13 LF isolates with 1 control strain.

Table 6 showed that in most of the cases, the zone diameter increased and in some cases, the zone diameter decreased and in few cases, no change was found. Hence, from this finding, it is assumable that metal resistant isolates, having negative or no effects on antibiotic resistance, could be used for bioremediation. Since these isolates can accumulate metals into their body (Medfu Tarekegn et al., 2020) and have decreased effects or no effect on resistance, it therefore means that

Table 6. Zone of inhibition of the representative LF isolates (n=14) to the heavy metals, Ni (0.6 mM), Cr (1.0 mM), Pb (0.6 mM), respectively in the presence of antibiotics.

Lab ID	AZM (diameter in mm)		SXT (diameter in mm)		CTX (diameter in mm)		TE (diameter in mm)		CIP (diameter in mm)	
	Ab	Ab + HM	Ab	Ab + HM	Ab	Ab + HM	Ab	Ab + HM	Ab	Ab + HM
23	10±0.06	(Ni) - (Cr) 7±0.03 (Pb) 12±0.07	7±0.03	(Ni) - (Cr) 7±0.02 (Pb) 7±0.01	22±0.23	(Ni) - (Cr) 30±0.27 (Pb) 26±0.23	7±0.02	(Ni) - (Cr) 10±0.05 (Pb) 12±0.08	20±0.12	(Ni) - (Cr) 12±0.06 (Pb) 10±0.07
27	7±02	(Ni) 20±0.16 (Cr) 7±0.04 (Pb) 10±0.08	7±0.01	(Ni) 7±0.04 (Cr) 7±0.02 (Pb) 7±0.03	20±0.17	(Ni) 30±0.31 (Cr) 30±0.29 (Pb) 30±0.28	7±0.04	(Ni) 7±0.04 (Cr) 10±0.05 (Pb) 10±0.06	24±0.15	(Ni) 24±0.21 (Cr) 20±0.23 (Pb) 22±0.18
31	20±0.15	(Ni) 26±0.21 (Cr) 24±0.25 (Pb) 26±0.23	7±0.04	(Ni) 20±0.13 (Cr) 16±0.15 (Pb) 20±0.14	8±0.05	(Ni) 20±0.18 (Cr) 20±0.17 (Pb) 20±0.21	8±0.06	(Ni) 18±0.11 (Cr) 24±0.20 (Pb) 24±0.21	7±0.02	(Ni) 22±0.20 (Cr) 22±0.17 (Pb) 24±0.25
35	14±0.9	(Ni) 20±0.17 (Cr) 7±0.03 (Pb) 18±0.15	7±0.02	(Ni) 7±0.04 (Cr) 7±0.02 (Pb) 7±0.03	22±0.12	(Ni) 26±0.25 (Cr) 24±0.20 (Pb) 30±0.32	20±0.14	(Ni) 18±0.16 (Cr) 26±0.23 (Pb) 20±0.17	24±0.17	(Ni) 24±0.26 (Cr) 30±0.32 (Pb) 24±0.21
39	10±0.07	(Ni) 20±0.14 (Cr) 7±0.03 (Pb) 26±0.21	7±0.03	(Ni) 14±0.11 (Cr) 12±0.10 (Pb) 14±0.12	24±0.15	(Ni) 16±0.14 (Cr) 14±0.12 (Pb) 20±0.18	7±0.03	(Ni) 16±0.15 (Cr) 24±0.14 (Pb) 20±0.16	18±0.21	(Ni) 22±0.19 (Cr) 22±0.24 (Pb) 24±0.20
46	16±0.11	(Ni) 20±0.25 (Cr) - (Pb) -	10±0.06	(Ni) 20±0.18 (Cr) - (Pb) -	10±0.03	(Ni) 20±0.19 (Cr) - (Pb) -	20±0.12	(Ni) 14±0.15 (Cr) - (Pb) -	20±0.19	(Ni) 22±0.25 (Cr) - (Pb) -
51	14±0.09	(Ni) 20±0.16 (Cr) 20±0.18 (Pb) 28±0.26	7±0.01	(Ni) 20±0.14 (Cr) 22±0.16 (Pb) 20±0.15	22±0.14	(Ni) 26±0.21 (Cr) 20±0.18 (Pb) 22±0.20	10±0.11	(Ni) 20±0.18 (Cr) 26±0.23 (Pb) 24±0.21	10±0.06	(Ni) 20±0.23 (Cr) 22±0.26 (Pb) 22±0.19
68	14±0.05	(Ni) 20±0.19 (Cr) 7±0.04 (Pb) 16±0.12	20±0.17	(Ni) 26±0.21 (Cr) 20±0.18 (Pb) 20±0.17	22±0.16	(Ni) 32±0.30 (Cr) 28±0.25 (Pb) 30±0.27	7±0.02	(Ni) 7±0.03 (Cr) 10±0.05 (Pb) 10±0.07	26±0.23	(Ni) 24±0.18 (Cr) 24±0.20 (Pb) 22±0.18
73	16±0.12	(Ni) 24±0.20 (Cr) 7±0.03 (Pb) 10±0.05	16±0.13	(Ni) 22±0.20 (Cr) 16±0.13 (Pb) 16±0.15	14±0.09	(Ni) 20±0.18 (Cr) 16±0.14 (Pb) 18±0.15	24±0.18	(Ni) 22±0.19 (Cr) 26±0.25 (Pb) 24±0.20	22±0.18	(Ni) 22±0.20 (Cr) 16±0.13 (Pb) 16±0.16
83	20±0.17	(Ni) 20±0.18 (Cr) 22±0.16 (Pb) 24±0.22	14±0.12	(Ni) 14±0.12 (Cr) 18±0.15 (Pb) 20±0.18	12±0.07	(Ni) 18±0.21 (Cr) 16±0.17 (Pb) 22±0.23	16±0.15	(Ni) 24±0.23 (Cr) 26±0.27 (Pb) 28±0.25	22±0.16	(Ni) 20±0.21 (Cr) 20±0.22 (Pb) 22±0.25
11*	28±0.22	(Ni) 25±0.21 (Cr) 26±0.23 (Pb) 28±0.22	23±0.21	(Ni) 20±0.14 (Cr) 25±0.20 (Pb) 23±0.21	28±0.28	(Ni) 19±0.21 (Cr) 22±0.18 (Pb) 22±0.20	24±0.21	(Ni) 15±0.10 (Cr) 28±0.32 (Pb) 28±0.29	25±0.21	(Ni) 26±0.27 (Cr) 27±0.30 (Pb) 25±0.26
85*	27±0.26	(Ni) 24±0.21 (Cr) 23±0.18 (Pb) 27±0.21	22±0.25	(Ni) 18±0.15 (Cr) 25±0.23 (Pb) 22±0.18	26±0.21	(Ni) 22±0.16 (Cr) 21±0.28 (Pb) 20±0.18	23±0.20	(Ni) 15±0.14 (Cr) 29±0.20 (Pb) 27±0.25	21±0.20	(Ni) 23±0.20 (Cr) 25±0.22 (Pb) 21±0.19
99*	26±0.24	(Ni) 24±0.24 (Cr) 24±0.20 (Pb) 26±0.23	20±0.11	(Ni) 18±0.13 (Cr) 22±0.16 (Pb) 20±0.18	30±0.26	(Ni) 20±0.19 (Cr) 18±0.16 (Pb) 22±0.25	22±0.17	(Ni) 14±0.10 (Cr) 26±0.29 (Pb) 26±0.26	22±0.16	(Ni) 24±0.26 (Cr) 22±0.29 (Pb) 22±0.27
E.coli ATCC (25922)	28±0.25	(Ni) 27±0.25 (Cr) 22±0.21 (Pb) 23±0.20	28±0.15	(Ni) 27±0.21 (Cr) 23±0.25 (Pb) 24±0.24	30±0.27	(Ni) 28±0.26 (Cr) 26±0.25 (Pb) 25±0.26	30±0.24	(Ni) 29±0.28 (Cr) 25±0.20 (Pb) 26±0.27	28±0.25	(Ni) 27±0.20 (Cr) 25±0.19 (Pb) 23±0.21

ID: Identification; CIP: Ciprofloxacin; CTX: Cefotaxime; AZM: Azithromycin; SXT: Sulfamethoxazole-trimethoprim; TE: Tetracycline; Ab: Antibiotic; HM: Heavy metal; mm: millimeter; Ni: Nickel; Cr: Chromium;Pb: Lead;11*, 85*, 99*: LF antibiotic sensitive isolates; ATCC (25922): Negative control strain.

they are not harmful to humans and animals if these isolates discharge into the environment.

The influences of Cr on AZM and CIP and also Pb on CIP were found most significant as in both cases the zone of inhibition decreased for most isolates. For ATCC (25922), the zone of inhibition decreased for all antibiotics

in the presence of Ni, Cr and Pb. By observing the 3 antibiotic sensitive isolates and 1 negative control strain, it was quite difficult to determine the actual effects of heavy metal to antibiotics for these isolate, because the patterns seem highly variable. In the present research work, the complexity in observed results mainly relied upon the types of various heavy metals, antibiotics, and their concentrations, which might be clarified by two major aspects; firstly, the occurring reactions due to the interactions between the chemical groups present on the heavy metals and antibiotics; and secondly, the particular biological effect caused by those. More particularly, the resultant concentrations of metals or antibiotic compounds might be impaired by the chemical group involving reactions among them (Zhang et al., 2012) and, therefore, if the ultimate end products of heavy metal and antibiotic reactions are highly toxic compared to those specific starting compounds, the bacteria might show weaker resistance to those antibiotic-derived substances (Tamilselvi and Mugesh, 2008).

Nevertheless, when the toxicity of the resultant end products is lower than starting toxic substances, the bacterial resistance properties to antibiotic-derived compounds could be unaltered or increased for the effectively reduced concentrations. Similarly, the presence of Cu or Zn can improve resistance to imipenem (a β-lactam antibiotic) in *Pseudomonas aeruginosa* due to possibly causing coagulation reaction (Caille et al., 2007). Hence, it is likely that the chemical reactions can be altered by various factors such as the categories and concentrations of metals, antibiotics, as well as, the presence of toxic contaminants in the same environmental system.

Moreover, the overall effects of heavy metals on bacterial antibiotic resistance attribute might additionally lead to the prodigy of bacterial cross-resistance and the pertinent mechanisms might be much complex. In general, heavy metals had a key role on the bacterial enzymatic activities and those trace elements are vital for the growth of bacteria (Liu et al., 2012), although it could be stress causing factors impairing the protein synthesis in *Pseudomonas fluorescens* (Sharma et al., 2006). The stress factors alter the bacterial efflux pumps and the integron-comprising mobile elements which causes cross-resistance among heavy metals and antibiotics (Petrova et al., 2011). The *Vibrio* species contain cryptic plasmids and these plasmids have important role in showing resistance to both Hg and antibiotics (Wang et al., 2006; Zhang et al., 2006). Further, the heavy metals in low concentrations could promote protein expression such as metallothionein, and the metallothionein synthesis has generally been noticed to be induced by high concentrations of a few metals and further antibiotic resistance might be inhibited rather than being enhanced (Strouhal et al., 2003). Although the underlying distinct mechanisms of heavy metal induced stress on microbial antibiotic resistance are still ambiguous due to the lack of enough evidences from related studies, relevant cross-resistance studies with a number of bacteria would be supportive in explaining the environmental turnover and the associated risks of metals and antibiotics.

Plasmid profiling of representative LF isolates (n=14)

Plasmid profiling (Figure 7) showed that all the isolates had various plasmid patterns ranging from P1 to P7. Seven isolates were found to contain one plasmid and 2 isolates contained 2 plasmids. Two sensitive isolates including one resistant isolate (resistant to only TE) and ATCC (25922) were plasmid less. Only one isolate was found to contain 5 plasmids. A large sized (21kb) plasmid was found in 6 isolates. Five isolates contained 12 Kb plasmids including one sensitive isolate. Only one isolate contained 3 plasmids. Smaller sized plasmids range from 5.5 to 1.6 Kb. In Table 7, plasmid size and patterns were given for 12 representative LF isolates. In Table 8, a summary of the characters of all 14 representative LF isolates including three susceptible isolates and one negative control strain was provided.

Malik and Jaiswal (2000) reported that increasingly polluted environment is responsible for the extent of the high populations of heavy metal resistant bacteria, and they also stated the high prevalence of plasmid-containing bacterial strains in polluted sites compared to unpolluted ones. In the present study, almost all the bacterial isolates displayed a remarkable degree of resistance to the applied antibiotics and heavy metals. In addition, nowadays, it is conclusively proved that the resistance features are generally located on the extra-chromosomal DNA widely known as plasmids (Collard et al., 1994; Dhakephalkar and Chopade, 1994; Guo et al., 2006). It was reported on a previous study that plasmids are responsible for multidrug resistance and generally found ≥12 Kb in size (Jacoby and Sutton, 1991; Parvin et al., 2014). Therefore, the outcomes of our study had similar results and strongly supported with these findings. Moreover, our experimental results are also supported by the findings of previously conducted studies on plasmid directed heavy metal resistance characteristics of isolates of bacteria collected from the environments contaminated by heavy metals (Vajiheh et al., 2003; Zolgharnein et al., 2007). Furthermore, it was noticed from our study that plasmid free bacterium displayed either resistance to heavy metals or antibiotics as one TE resistant isolate did not contain any plasmid. As a result, it clearly suggests that the distinct features associated with antibiotics and heavy metal resistance detected in different isolates of bacteria related to the plasmid and/or chromosomal DNAs. Although, a broad range of plasmid DNAs are found in bacterial isolates from textile effluent contaminated environment, there was no constant correlations between antibiotic resistance property and plasmid profiles. This attribute is not unexpected because

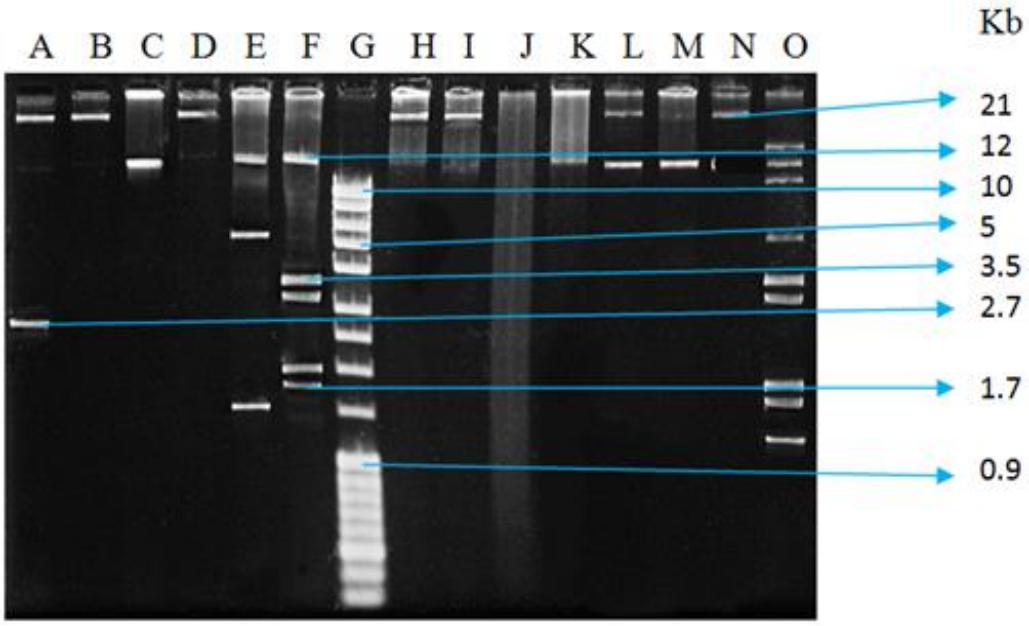

Figure 7. Plasmid profiles of representative LF isolates (n=12) (Lane A: 23; Lane B: 27, Lane C: 31, Lane D: 35, Lane E: 39, Lane F: 46; Lane G: Circular DNA Ladder; Lane H: 51, Lane I: 59, Lane J: 68; Lane K: 73, Lane L: 83, Lane M: 99 (antibiotic sensitive), Lane N: R1, Lane O: V-517).

Table 7. Plasmid patterns of representative LF isolates (n=14) showing resistance to both heavy metals and antibiotics.

Lab ID	Isolate ID	Lanes in Figure 7	Plasmid size in base pair (kb)	Plasmid pattern (P)
23	S2L1 LF3	Lane A	21, 2.7	P1
27	S2L2 LF3	Lane B	21	P2
31	S2L3 LF3	Lane C	12	P3
35	S2L4 LF3	Lane D	21	P2
39	S2L5 LF3	Lane E	12, 5.5, 1.6	P4
46	S3L2 LF2	Lane F	12, 3.5, 3.3, 2.0, 1.7	P5
51	S3L3 LF3	Lane H	21	P2
59	S3L5 LF3	Lane I	21	P2
68	S4L2 LF4	Lane J	Plasmid less	P6
73	S4L4 LF1	Lane K	12	P7
83	S5L1 LF3	Lane L	21, 12	P8
11*	S1L3 LF3		Plasmid Less	P6
85*	SFL2 LF1		Plasmid Less	P6
99*	S5L5 LF3	Lane M	12	P3
E.coli	ATCC (25922)		Plasmid less	P6
	V 517	Lane O	14,12,10,5,3.5,3,1.7,1.6,1.0	
	R 1	Lane N	21	

ID: Identification; SL: Sample location; LF: Lactose fermenter; 11*, 85*, 99*: Antibiotic sensitive LF isolates; R1: Reference strain; V517: Reference strain; P: Pattern.

the similar pattern of antibiotic resistance can be concealed by unconnected plasmid, transposon, phage and chromosomal genes (Jain et al., 2009; Vajiheh et al., 2003). Therefore, the antimicrobial resistance motifs and plasmid profiles are consistently insufficient to explain the connections between distinctive isolates of bacteria from

Table 8. Representative LF isolates (n=14) showing susceptible pattern to heavy metals and antibiotics in relation to their plasmid profiles.

Lab ID	Sample ID	Antibiotic susceptibility test			Maximum tolerable conc. to heavy metals (mM)			In the presence of maximum tolerable conc. to heavy metal, the changes of zone of inhibition of antibiotics measured in mm						Plasmid profiling (size in Kb)
								Ni (0.3 mM)		Cr (1.0 mM)		Pb (0.6 mM)		
		R	I	S	Ni	Cr	Pb	E	D	E	D	E	D	
23	S2L1 LF3	AZM,SXT,TE	CTX,CIP	-	0.6	1.0	0.6	-	-	-	-	-	-	21, 2.7
27	S2L2 LF3	AZM,SXT,TE	CTX	CIP	0.3	1.0	0.6	AZM,CTX	-	CTX,TE	AZM,SXT,CIP	AZM,CTX,TE	CIP	21
31	S2L3 LF3	SXT,CTX,CIP,TE	-	AZM	0.3	1.0	0.6	All	-	All	-	AZM,SXT,CTX,TE,CIP	-	12
35	S2L4 LF3	SXT	AZM,CTX	TE,CIP	0.3	1.0	0.6	AZM,SXT,CTX	TE	CTX,TE,CIP	AZM	AZM,CTX	-	21
39	S2L5 LF3	AZM,SXT,TE	CIP	CTX	0.3	1.0	0.6	AZM,SXT,TE,CIP	CTX	SXT,TE,CIP	AZM,CTX	AZM,SXT,TE, CIP	CTX	12, 5.5, 1.6
46	S3L2 LF2	SXT,CTX	AZM,CIP	TE	0.3	2.0	1.2	AZM,SXT,CTX, CIP	TE	-	-	-	-	12, 3.5, 3.3, 2.0, 1.7
51	S3L3 LF3	SXT,TE,CIP	AZM,CTX	-	0.3	1.0	0.6	All	-	AZM,SXT,TE,CIP	CTX	AZM,SXT,TE, CIP	-	21
59	S3L5 LF3	All	-	-	0.6	2.0	1.2	-	-	-	-	-	-	21
68	S4L2 LF4	TE	AZM,CTX	SXT,CIP	0.3	1.0	0.6	AZM,SXT,CTX	CIP	CTX,TE	AZM,CIP	AZM,CTX,TE	CIP	Plasmid less
73	S4L4 LF1	CTX	AZM	SXT,TE, CIP	0.3	1.0	0.6	AZM,SXT,CTX	TE	CTX,TE	AZM,CIP	CTX	AZM, CIP	12
83	S5L1 LF3	CTX	SXT,TE	AZM,CIP	0.3	1.0	0.6	CTX,TE	CIP	AZM,SXT,CTX,TE	CIP	AZM,SXT,CTX,TE	-	21, 12
11*	S1L3 LF3	-	-	All	0.3	1.0	0.6	-	-	-	-	-	-	Plasmid Less
85*	SFL2 LF1	-	-	All	0.3	1.0	0.6	-	-	-	-	-	-	Plasmid Less
99*	S5L5 LF3	-	-	All	0.3	1.0	0.6	CIP	AZM,SXT, CTX,TE	SXT,TE	AZM,CTX	TE	CTX	12
E.coli	ATCC (25922)	-	-	All	-	-	-	-	-	-	-	-	-	Plasmid less

ID: Identification; R: Resistance, I: Intermediate, S: Sensitive, CIP: Ciprofloxacin; CTX: Cefotaxime; AZM: Azithromycin; SXT: Sulfamethoxazole-trimethoprim; TE: Tetracycline; Conc.: Concentration; Ni: Nickel; Cr: Chromium; Pb: Lead; E: Enhanced; D: Decreased, Kb: Kilo base pair, 11*, 85*, 99*: *A*ntibiotic sensitive LF isolates, ATCC (25922): *E. coli* negative control strain.

polluted environment and may lead to flawed epidemiologic conclusions. In summary, these significant bacterial isolates from textile industrial effluents had a greater range of resistance property against different antibiotics and heavy metals, as well as, found to contain an extra-chromosomal or plasmid DNA of 12 Kb and 21 Kb.

Conclusion

The results obtained from this study suggest that industrial effluents are enriched organic medium for supporting the potentially high growth and spreading of microorganisms, which are ultimately resistant to different antibiotics and heavy metals. Thus, environmental distributions of such bacteria are putting a deliberate threat for successfully treating the infectious diseases in some cases. In most cases, heavy metal exerts negative or no effects on antibiotic resistance of isolates. The heavy metal resistance patterns are of comparatively minor clinical concern than antimicrobial resistance; nevertheless, the obtained knowledge about heavy metal resistance may administer

some valuable evidences of antibiotic resistance, genetic study of plasmid, physiological and ecological features of bacteria inhabited in polluted water and soil environments. In summary, this study gives a general view of the state of lactose fermenting isolates growing in heavy metal enriched context to understand the aftereffects of discharging such effluents into the environment. Therefore, it is very essential to investigate the complete properties of heavy metal and antibiotic resistant bacterial isolates both phenotypically and molecularly to combat possible health hazards caused by the infection of such isolates.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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