INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 8, No. 5, p. 87-95, 2016

RESEARCH PAPER OPEN ACCESS

Antimicrobial resistance pattern of some selected bacteria isolated from Hatirjhil lake water

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Key words: Wastewater, Total aerobic plate count, Coliform, Antimicrobial activity.

http://dx.doi.org/10.12692/ijb/8.5.87-95 Article published on May 18, 2016

Abstract

The greatest microbial risks are associated with ingestion of water that is contaminated with human or animal feces. Wastewater discharges in fresh water and coastal seawaters are the major source of faecal microorganism, including pathogens.The purpose of our study is to analyze antimicrobial resistant pattern of bacteria isolated from hatirjhil lake against commercial antibiotics.Hatirjhil lake water was collected and analyzed. Number of total aerobic plate count was high and pathogenic organism such as Coliform, *Salmonella, Vibrio cholerae, Staphylococcus aureus, Escherichia coli* were found. Antimicrobial activity of isolatedbacteriawere seen by modified Kirby Bauer disc diffusion methodagainst *9 commercial antibiotics*. . Among 9 commercial antibiotics Imipenemand Cholaramphenicol showed almost 100% sensitivity against *Salmonella* and*Staphylococcus aureus*.Imipenem and Amikacin showed92.31% sensitivity against*Escherichia coli*.Imipenem showed76.92% sensitivity against *Vibrio cholerae*. This study was an indication that organisms are getting resistant day by day against commercial antibiotics.So it is necessary to take attempt for continuous monitoring of antibiotic susceptibility of bacteria before antibiotic prescription.

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Introduction

Water has always been a prime item for human life as two thirds of the earth surface is covered by water.Now water is considered as a risk factor into two basic categories- chemical and biological, which is subjected to pollution. Bangladesh had always been predominantly and agricultural based country and in early days pollution was never felt in this region. Since early sixties, of necessity, industries of various kinds started to grow up slowly. Due to the discharge of various industrial wastes into water bodies, ecological imbalanceincreases continuously (Diffusion pollution conference Dublin, 2003). Hatirjhil is a commercial area, located at the center of the capital city of Dhaka, Bangladesh. It is considered as the center of skyscraper boom in Bangladesh (The Financial Expess 2015). But for this crowded capital, this spectacular achievement has been undermined by the severely polluted water in the lake. Its water emanates so strong stinks that people do not only using its walkways but also the nearby residents are all overwhelmed by nausea (Dhaka Mirror, 2013). In rainy season, solid waste mixed with it, enters the Hatirjhillake and thereby, pollutes the lake water. Most of the visitors leave bottles, polybags, biscuit, newspaper and thus environment also get polluted.The lake water contaminated by human and animal wastes contains a variety of bacterial, viral and protozoan pathogens (Ali *et al.*, 2012).

The increased re-use of wastewater raises concerns about the occurrence and survival of pathogens in the environment. The presence of pathogenic enteric micro-organisms in aquatic environments can be a source of disease when water is used for drinking, recreational activities or irrigation. The sanitary risk is increased if the pathogenic enteric bacteria present in waters are antibiotic resistant because human infections caused by such bacteria could be difficult to treat with drugs. In addition, fecal bacteria might be able to transmit antimicrobial resistance to autochthonous bacteria through lateral transfer, when the resistance genes are carried by transferable and mobile genetic elements such as plasmids and thus contributing to the spread of antimicrobial resistance (Nyamboya*et al.*, 2013).

The Total Bacterial count (TBC) is widely used test parameter to indicate the microbiological quality of water. Coliform group of bacteria that have a wide distribution in sewage and polluted water environment; is an indicator for the assessment of faecal pollution (Ali *et al.*, 2012).*Escherichia coli* is a coliform organisms, are generally live longer in water.*Escherichia coli* has a great impact on public health, with an economic cost of several billion dollars annually (Russo *et al.*,2003). The treatment of *E. coli* infections is increasingly becoming difficult due to development of resistance against antibiotics. Therefore, it is necessary to know the antibiotic susceptibility pattern of pathogenic *E. coli* to select the correct antibiotic for the proper treatment of the infections (Sharma *et al.*,2007).

Staphylococcus aureus remains a potent human pathogen, since it is one of the most common cause of nosocomial as well as community acquired infection. It is also the most significant pathogen known for causing sporadic infections and epidemics. Most of the *S. aureus* infections are caused by Methicillin-sensitive *S. aureus* strains (MSSA) that are usually susceptible to major classes of anti-staphylococcal antibiotics. In the recent years, the widespread use of antibiotics has undoubtedly accelerated the evolution of MRSA and led to the emergence of strains that have systematically acquired multiple resistance genes (Pandey *et al.*,2012).

The survival and recovery of *Salmonella* species from surface water, wastewater and bottled water have been investigated. *Vibrio* species have also been reported in drinking water, surface water and sewage. *Salmonella* species is dangerous water borne bacterial pathogen in terms of human health and diseases. Various serotypes of *Salmonella* species have reportedly been responsible for water and food borne epidemics in various countries emphasizing the importance of the pathogen as a food safety concern. Water is also an important source for human infections with antimicrobial resistant *Vibrio* species

(Nyamboya*et al.*,2013).

The primary objective of our study was to assess the water quality of Hatirjhillake in Dhaka city. The study was also done to determine the antimicrobial resistance of isolated indicator organisms such as *Escherichia coli, Staphylococcus aureus, Vibriocholerae and Salmonella* species.

Materials and methods

Sample collection

In the present study 11 waste water mixing Point of Hatirjhil Lakewere marked. The waste water samples were collected in sterile glass bottles and transferred from the sites to the laboratory in ice box.

Total Bacterial Count (TBC)

Collected water samples were analyzed for total bacterial count (TBC) using pour plate technique. Method for decimal dilution of water samples was used for the determination of total bacterial load on nutrient agar medium (APHA, 2005). The plates were incubated for 24 hours at 37°C.

The most probable number technique (MPN) andEscherichia coli

The most probable number technique was carried out for estimation of some microbial indicators in the collected water samples using special presumptive and confirmed tests for each indicator. During presumptive test, 5ml of each appropriate three decimal dilutions of collected water samples were used to inoculate five tubes each containing 5ml of proper medium (single strength), and the tubes were incubated at 37ºC for 48 hours.

The positive presumptive tubes were used to inoculate the confirmed test which detected the bacterial indicators as following:-Total coliform; Lauryl tryptose broth medium was used for presumptive test. The positive tubes which showed gas and acid were used to inoculate brilliant green lactose bile broth medium (BGB), as a confirmed test. The production of gas and acid was recorded as positive confirmed test for total coliforms (APHA, 2005).

Faecal coliform estimation was carried out by inoculation in the EC broth tubes from positive BGB broth medium tubes, then incubated at 44.5ºC for 24 hours (APHA, 2005). The positive tubes containing gas production was used to detect the count per 100 ml sample (MPN index/100ml) and streak the eosin methylene blue agar medium (EMB) plates, then incubated at 37ºC for 24 hours. Metallic sheen colonies considered as a positive confirmed results for *E. coli* presence (APHA, 2005).

*Staphylococcus aureus*sp.

Staphylococcus *aureus*sp was determined by streaking one loop from buffer peptone water tubes on the surface of mannitol salt agar plates, then incubated at 37ºC for 24 hours. The suspected colonies had yellow zones(APHA, 2005).

Salmonella sp.

Salmonellae groups were counted from inoculated buffer peptone water tubes (BSI, 2002). One loopful growth from these tubes was streaked on Bismuth sulphite agar plate. After incubation at 37ºC for 48 hours, typical black colonies with or without metallic sheen and blackening extended beyond the colonies considered as confirmed positive results for the presence of Salmonellae.

*Vibrio cholerae*sp.

Sample was enriched in Alkaline peptone water and incubated 37ºC for 6-8 hours. A loopful growth was then streaked to thiosulphate citrate bile salt sucrose (TCBS) agar plate. After 24 hour incubation, distinctive yellow colonies on agar plates were suspected as *Vibrio cholerae*species. Then the isolates were purified several times on TCBS agar plates.

Antimicrobial susceptibility test

The antimicrobial susceptibility pattern of all the four isolated strains- *Escherichia coli, Staphylococcusaureus, Salmonella* and *Vibriocholerae*was determined by modified Kirby Bauer disc diffusion method against the following antibiotics: Imipenem(IPM), Amikacin(AK), Ciprofloxacin(CIP), Tetracycline(TE), Ceftazidime(CAZ), Cefuroxime(CXM), Azithromycin(AZM), Ceftriaxone(CTR) and Chloramphenicol(C).

Results

In this present study 11 waste water points of Hatirjhillake were selected. The samples were analyzed in the following parameters- Total bacterial count (TBC), Total coliform (MPN/100ml), *Escherichia coli* (MPN/100ml), *Staphylococcus aureus* (cfu/ml), *Salmonella* spp./25ml and *Vibrio cholerae*/25ml. Total bacterial count (TBC) and Total coliform count (MPN/100ml) were presented in Table 1.Total bacterial count (TBC) was high in all selected water points. Coliform is >2400 MPN/100ml in all samples.

Table 1. Total Microbial load of Hatirjhil lake water.

Serial number	Total Bacteria Count (TBC)	Total coliform (MPN/100ml)
1	5×10^6	>2400
2	8×10^6	>2400
3	5×10^7	>2400
4	6×10^6	>2400
5	4×10^6	>2400
6	4×10^5	>2400
7	11×10^4	>2400
8	7×10^6	>2400
9	11×10^5	>2400
10	9×10^7	>2400
11	5×10^6	>2400

Table 2. Antimicrobial susceptibility patterns of *E. coli* isolates against commercial antibiotics.

Name of antibiotics	Antibiotic conc.	Sensitivity pattern of isolated *E. coli*		
		% R	% I	% S
Imipenem (IPM)	10	7.69	0	92.31
Amikacin (AK)	25	7.69	0	92.31
Ciprofloxacin (CIP)	30	76.92	0	23.08
Tetracycline (TE)	25	100	0	0
Ceftazidime (CAZ)	30	100	0	0
Cefuroxime (CXM)	30	53.85	0	46.15
Azithromycin(AZM)	05	76.92	23.08	0
Ceftriaxone (CTR)	10	30.77	15.38	53.35
Chloramphenicol (C)	30	30.77	7.69	61.54

Escherichia coli, *Staphylococcus aureus*, *Salmonella* spp.and *Vibrio cholerae* were present in all samples. 13 bacteria of each species were selected for antimicrobial study. Nine types of commercial antibiotics which are frequently used against enteric diseases; were investigated against selected organisms and the sensitivity of tested organisms against antibiotics were compared to the standard zone diameter (Table 6) of these organisms against these antibiotics (Watts *et al.*,2008).

Table 3. Antimicrobial susceptibility patterns of *Salmonella* spp. isolates against commercial antibiotics.

Name of antibiotics	Antibiotic conc.	Sensitivity pattern of isolated *Salmonella* spp.		
		% R	% I	% S
Imipenem (IPM)	10	0	0	100
Amikacin (AK)	25	0	30.77	69.23
Ciprofloxacin (CIP)	30	30.76	38.46	30.76
Tetracycline (TE)	25	100	0	0
Ceftazidime (CAZ)	30	76.92	23.08	0
Cefuroxime (CXM)	30	53.85	0	46.15
Azithromycin(AZM)	05	92.31	7.69	0
Ceftriaxone (CTR)	10	46.15	7.69	46.15
Chloramphenicol (C)	30	0	0	100

Isolated *E. coli* showed highest 92.31% sensitivity against Imipenem (10µg/disc) and Amikacin (25µg/disc). E. coli was not sensitive against Tetracyciln(25µg/disc), Ceftazidime (30µg/disc) and Azithromycin (5µg/disc) (Table 2).

Salmonella spp. showed 100% sensitivity against Imipenem (10µg/disc) and Cholamphenicol (30µg/disc). On the other hand *Salmonella spp.* was 100% resistant against Tetracyciln (25µg/disc)(Table 3).Same results found in case of *Staphylococcus aureus* (Table 4).

Table 4. Antimicrobial susceptibility patterns of *Staphylococcus aureus* isolates againstcommercial antibiotics.

Name of antibiotics	Antibiotic conc.	Sensitivity pattern of isolated *S. aureus*		
		% R	% I	% S
Imipenem (IPM)	10	0	0	100
Amikacin (AK)	25	0	23.08	76.92
Ciprofloxacin (CIP)	30	30.77	15.38	53.35
Tetracycline (TE)	25	100	0	0
Ceftazidime (CAZ)	30	69.23	30.76	0
Cefuroxime (CXM)	30	46.15	7.69	46.15
Azithromycin(AZM)	05	76.92	23.08	0
Ceftriaxone (CTR)	10	69.23	0	30.76
Chloramphenicol (C)	30	0	0	100

Table 5. Antimicrobial susceptibility patterns of *Vibrio cholerae* isolates against commercial antibiotics.

Name of antibiotics	Antibiotic conc.	Sensitivity pattern of isolated *Vibrio cholera*		
		% R	% I	% S
Imipenem (IPM)	10	0	23.08	76.92
Amikacin (AK)	25	30.77	23.08	46.15
Ciprofloxacin (CIP)	30	84.62	15.38	0
Tetracycline (TE)	25	100	0	0
Ceftazidime (CAZ)	30	100	0	0
Cefuroxime (CXM)	30	76.92	15.38	7.69
Azithromycin(AZM)	05	100	0	0
Ceftriaxone (CTR)	10	53.85	15.38	30.77
Chloramphenicol (C)	30	0	30.77	69.23

Isolated *Vibrio cholerae*showed highest 76.92% sensitivity against Imipenem (10µg/disc). *Vibrio cholerae*was 100% resistant against Tetracyciln (25µg/disc), Ceftazidime (30µg/disc) and Azithromycin (5µg/disc) (Table 5).

Discussion

One of the major interests of the research was to find out any possible differences among the isolates of bacteria using antibiotic sensitivity pattern.Bacteria can defend themselves from the action of antibiotics by producing various metabolites that either degrade antibiotics or help the bacteria to survive by various mechanisms. Bacteria isolates subjected to antibiotic sensitivity testing against 9 most commonly prescribed antibiotics belonging to different groups as the study of antimicrobial resistance has been a major driving force in the understanding of many genetic and biochemical process in bacterial cells.

In addition, it has direct clinical value in making more effective use and development of antibiotics.

Table 6. Standard for clear zone diameters for Enterobacteriaceae.

Name of the antibiotics	Zone Diameter (mm)		
	Resistant (R)	Intermediate (I)	Sensitive (S)
Imipenem (IPM)	≤13	14-15	≥16
Amikacin (AK)	≤13	14-16	≥17
Ciprofloxacin (CIP)	≤15	16-20	≥21
Tetracycline (TE)	≤11	12-14	≥15
Ceftazidime (CAZ)	≤17	18-20	≥21
Cefuroxime (CXM)	≤14	15-17	≥18
Azithromycin(AZM)	≤13	14-17	≥18
Ceftriaxone (CTR)	≤13	14-20	≥21
Chloramphenicol (C)	≤12	13-17	≥18

From the study it was found that out of 9 antibiotics examined against isolates were resistant to 3 or more antibiotics and sensitive to 5 or more antibiotics. Tetracycline, Ceftazidime, and Azithromycin showed 100% resistance against all tested isolated bacteria and Ciprofloxacin showed 100% resistance against *Vibrio cholerae*. Third generation Cephalosporin such as Ceftazidime and Ceftrioxne indicate that these organism can possess ESBLs genes which is common phenomenon for acquiring resistance traits. Further studies require for molecular characterization of these isolated enteric pathogens. Cephalosporin resistance along with co-resistance of Ciprofloxacin now-a-days is common.

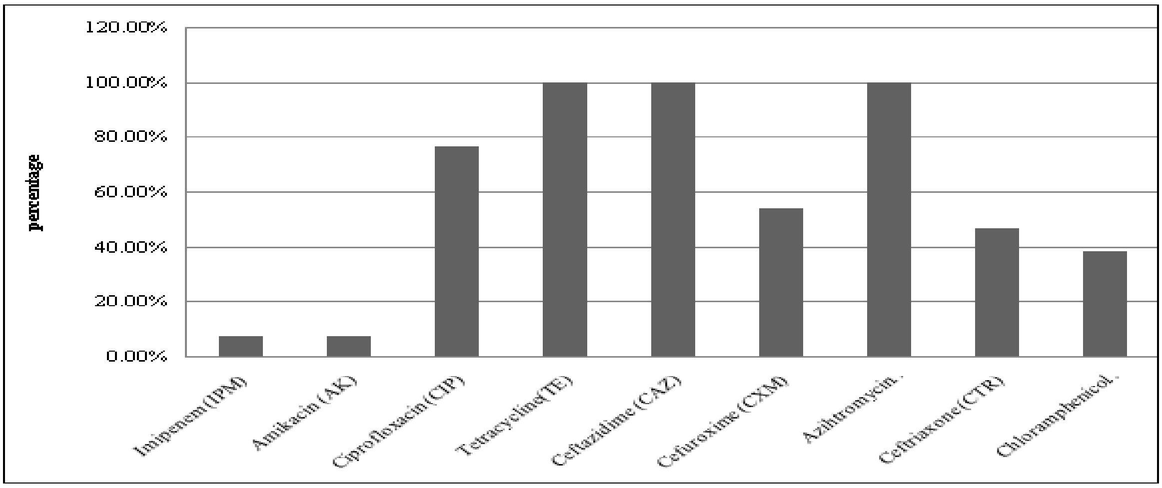

Fig. 1. Resistant percentage of *Escherichia coli*.

In a previous study carried out by Shahriar*et al.*,2010, 16.25%, 7.5%, 25% sensitivity of Ceftazidime, Ciprofloxacin and Tetracycline were found against *E.coli.*However, the present study showed a high alarming condition where Tetracycline and Ceftazidime are 100% resistance to isolated *E.coli*(Fig. 1). The second highest resistant holder isolates were resistant to Azihtromycin (76.92%) and Ciprofloxacin (76.92%) shown in Fig. 1.

Multi drug resistance was seen in most of the isolates and there was no common resistance pattern among the isolates. Such high incidence of multidrug resistance may presumably be due to indiscriminate use of antibiotics at the present time, which may eventually supersede the drug sensitive microorganisms from antibiotic saturated environment. The mechanism for the spread of antibiotic resistance must be considered seriously in a population like ours.

GordanaMijovic*et al., 2012*, in his study found that in 2005 the antibiotic susceptibility of *Salmonella* isolates were 91.9%, 98.9% 99.5% 99.5% to Ampicillin, Ceftriaxone, Ciprofloxacin, Chloramphenicol respectively and in 2010 the antibiotic susceptibility of *Salmonella* isolates were 92.9%, 97.1% 100%, 100% to Ampicillin, Ceftriaxone, Ciprofloxacin, Chloramphenicol respectively. But in our study we found that the antibiotic susceptibility of *Salmonella* isolates decrease to 30.76%, 46.15% to Ciprofloxacin and Ceftriaxone.

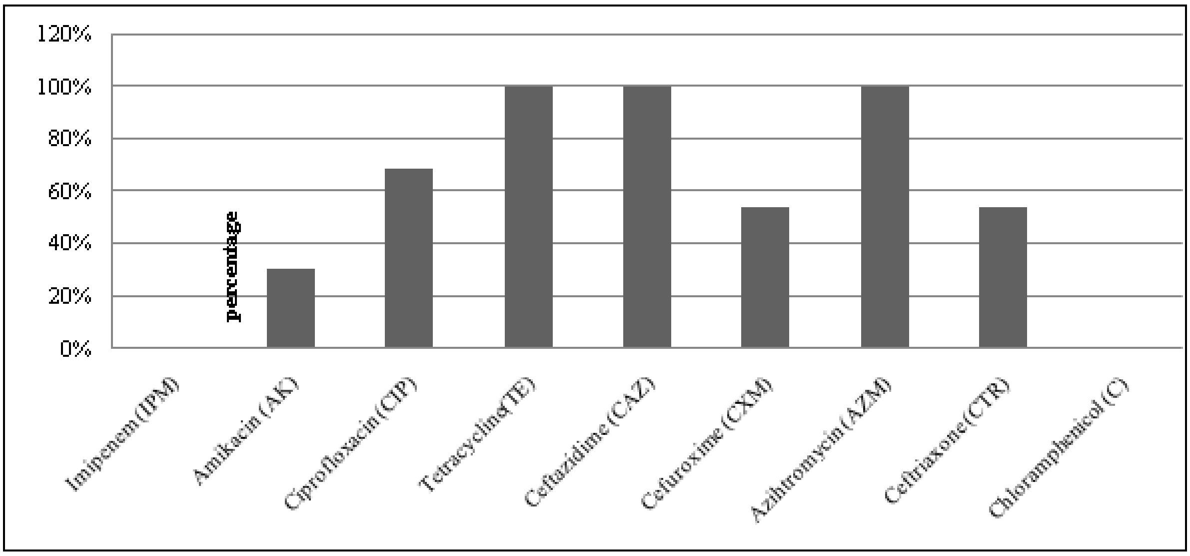

Fig. 2. Resistant percentage of *Salmonella* sp.

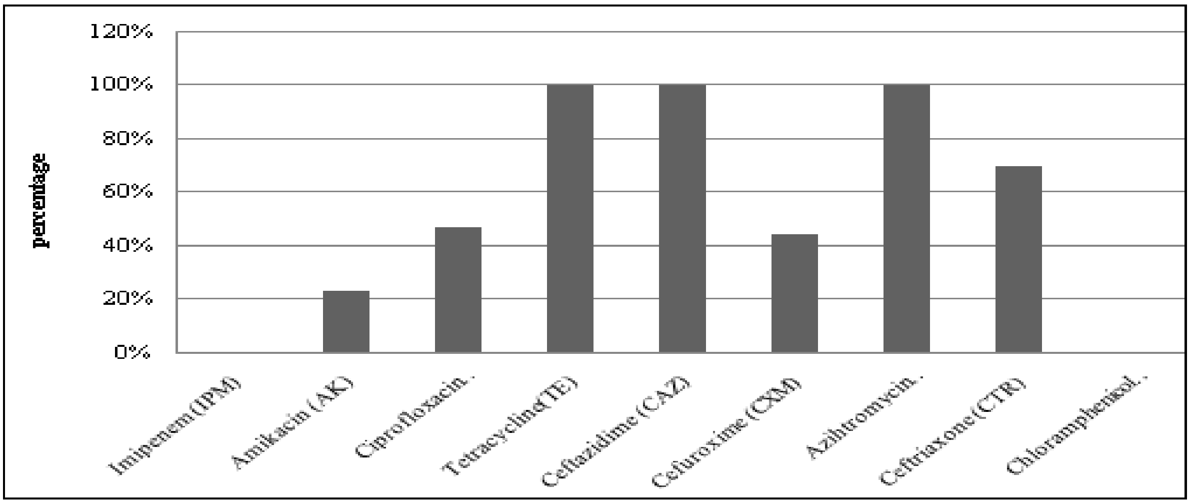

Fig. 3. Resistant percentage of *Staphylococcus aureus*.

The others antibiotic except Imipenem(100%) and Chloramphenical(100%) are getting resistance day by day. Amikacin and Cefuroxime showed 69.23% and 46.15% sensitivity against *Salmonella* isolates (Fig. 2). Tetracycline, Ceftazidime and Azihtromycin are fully resistance to *Salmonella* isolates which is very threat full to us. This result suggests that resistance to these antibiotics is increasing in recent years.

This study showed that *Staphylococcus aureus*are fully sensitive(100%) to Imipenem and Chloramphenicol and fully resistance to Tetracycline, Ceftazidime and Azihtromycin (Fig. 3). 76.92%, 53.35%, 46.15%, and 30.76% sensitivity showed to Amikacin, Ciprofloxacin, Cefuroxime and Ceftriaxone respectively. Biswajit Batabyal *et al* 2012 found *Staphylococcus aureus* was 70.5% sensitive and 29.5% resistant to imipenem. *Staphylococcus aureus* was 17.6% sensitive and 82.4% resistant to Ciprofloxacin.

In Bangladesh, children with invasive diarrhoea are normally treated with parenteral Ceftriaxone. It was interesting that most of our isolates were resistant to Ceftriaxone (53.85%). These results showed the need for new, affordable, and safe oral antimicrobial drugs to treat enterobacterial infections in children. If the current practice of the use of antibiotics is uphold, emergence of resistant *Vibrio cholerae*strains to this useful drug will follow and soon there will be no drug to treat life threatening systemic infections caused by resistant strains.

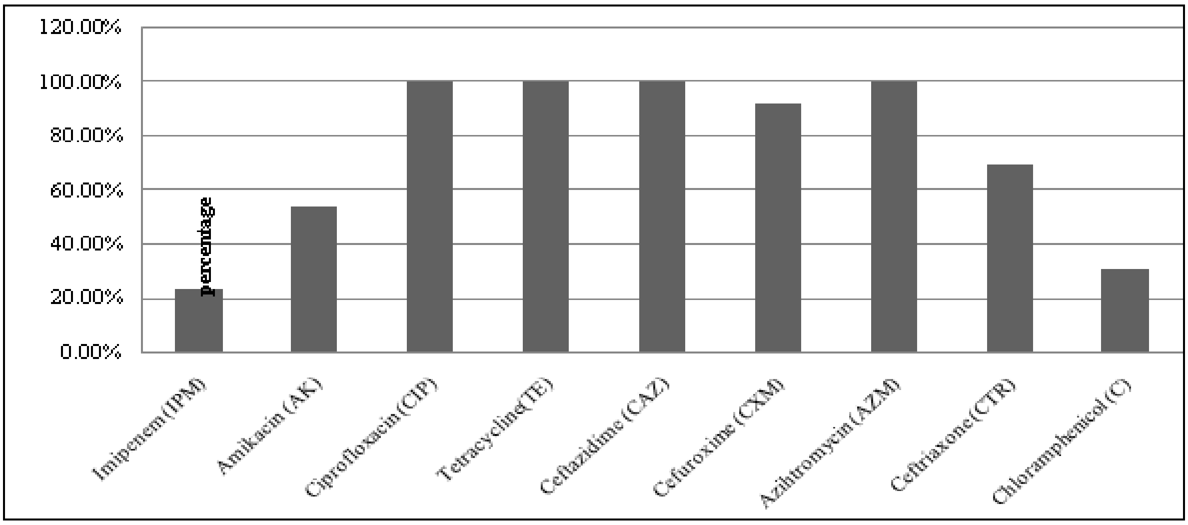

Fig. 4. Resistant percentage of *Vibrio cholerae*.

A study carried out by Shukla Das *et al.,*2008, during 2001-2006 showed that *Vibrio cholerae*strains are getting resistance to most of the antibiotics by which diarrhoea were treated. While in our study *Vibrio cholerae*strains showed 84.62%, 100%, 100%, 76.92%, 100% resistance to Ciprofloxacin, Tetracycline, Ceftazidime, Cefuroxime and Azihtromycin respectively (Fig. 4).

In this study some potent drugs have been found against the bacteria isolates. The most effective antibiotics to be used against bacteria isolates were Chloramphenicol, Imipenem and Amikacin.

Conclusion

This study was an indication that organisms are getting resistant day by day against commercial antibiotics. Antibiotics like Ciprofloxacin, Tetracycline, Azithromycin, Ceftazidime which were very effective against disease causing bacteria.Now-a-days most of these antibiotics are fully resistance against bacteria. This might be due to the selection pressure and genetic mutation of the organisms. So it is necessary to take attempt for continuous monitoring of antibiotic susceptibility of bacteria before antibiotic prescription.

Acknowledgement

This study was supported by the Food Microbiology laboratory under the Institute of Food Science and Technology (IFST), Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

References

Ali MS, Osman GA. 2012.Microbial load as pollution indicator in water of El-Khadralake at Wadi El-Natroun, Egypt. International journal of Agriculture and Environment **4,** 41-48.

APHA [American public health association] 2005. Standard Methods for the Examination of Water and Wastewater 21th ed. APHA, Inc. Washington, DC.Aquatic ecology and dangerous substances: Bangladesh perspective. Diffusion pollution conference Dublin 2003. Institute for Environmental and Developmental studies.

Biswajit Batabyal, Shibendu Biswas, Sukanta

Chakraborty, Priti D. deshal and Navonil de sarkar.OCT-DE2012. Prevalence and drug sensitivity pattern of Staphylococcus aureus in post-operative surgical oral and maxillofacial infections. International journal of life science &pharma research **2(4)**, L150-L158.

British standard institute (BSI), 2002. BSEN ISO 6579 incorporating corrigendum. No. 1. Microbiology of food and animal feeding stuffs-horizontal method for the detection of Salmonella spp. London:BSI.

Gordana Mijovic, Bogdanka Andric, Dragica Terzic, Milena Lopicic, Brankica Dupanovic. 2012. Antibiotic susceptibility of Salmonella spp.: A comparison of two surveys with a 5years interval.Journal of IMAB - Annual Proceeding (Scientific Papers) **18**, book 1. http://dx.doi.org/10.5272/jimab.2012181.216

Nyamboya Rosemary Atieno, Okemo Paul Owuor, Ombori Omwoyo.July 2013. Isolation of High Antibiotic Resistant Fecal Bacteria Indicators, Salmonella and Vibrio Species from Raw Abattoirs Sewage in Peri-Urban Locations of Nairobi, Kenya. Greener Journal of Biological Sciences ISSN: 2276-7762 **3(5),** 172-178.

Pandey S, Raza MS, Bhatta CP. April, 2012. Prevalence and antibiotic sensitivity pattern of Methicillin resistant- Staphylococcus aureus in Kathmandu Medical College-Teaching hospital. Journal of Institute of Medicine **34, 1** 13-17.

Polluted water of Hatirjheel, Dhaka, Tuesday, August. 2015. The Financial Express **22,** 272 REGD No DA 1581.

Russo TA, Johnson JR. 2003. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Microbes Infection. **5**: 449-56.

Shahriar Mohammad, Hossain Mahboob, Kabirshaila. Sep 2010. A survey on antimicrobial sensitivity pattern of different antibiotics on clinical isolates of Escherichia coli collected from Dhaka city, Bangladesh **14(3),** 19-20.

Sharma S, Bhat GK, Shenoy S. 2007. Virulence factors and drug resistance in Escherichia coli isolated from extra-intestinal infections. Indian J Med Microbiol **25,** 369-73.

Shukla Das, Rumpa Saha, Iqbal R, Kaur. Trend of antibiotic resistance of *Vibrio cholerae*strains from East Delhi. Indian J Med Res 127, May 2008, 478-482 P. Water polluted : Waste piles up: Dhaka Mirror, October 7, 2013.

Watts JL, Clinical Institute LS. 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard: National Committee for Clinical Laboratory Standards.