Prevalence and Diversity of Antimicrobial Resistant Pathogens From Medical and Community Wastewater in Dhaka City

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Declaration

It is hereby declared that

1. The thesis report submitted is my/our own original work while completing degree at Brac University.

2. The thesis report does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The thesis report does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I/We have acknowledged all main sources of help.

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Naima Hasan Bulbuli, Rownak Showkat Awysharjaw, Tabassum Lohani

May, 2022

APPROVAL

The thesis/project titled "Prevalence and Diversity of Antibiotic Resistant Pathogenic Bacteria from Hospital and Community Wastewater in Dhaka City" is submitted by **Naima Hasan Bulbuli (ID 18126058), Rownak Showkat Awysharjaw (ID 18126057)** and **Tabassum Lohani (ID 18126015)** of Fall 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology.

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Abstract:

Background: Uncontrolled and excessive exposure of antibiotics in the medical waste poses a selective pressure to acquire and disseminate highly antibiotic resistance genes to common pathogenic bacteria. This study is aimed to isolate common pathogenic bacteria and prevalence of multiple drug resistance genes in community and hospital wastewater.

Materials and Methods: Ten different wastewater samples (5 hospitals and 5 community wastewater) were aseptically collected and bacteriological investigations were performed for quantification and identification of bacterial isolates. Antibiotic susceptibility was performed by the Kirby-Bauer disk diffusion method and interpreted using the CLSI guideline. Polymerase-chain-reaction (PCR) will be performed for identification of ESBL and carbapenem resistance genes.

Results: Out of 234 isolates collected (based on different morphology); Gram-negative bacteria were predominant (85.04%). Top pathogenic-bacteria isolates were *E.coli*-(15%); *Klebsiella pneumonia* (12.82%); *Pseudomonas aeruginosa* (8.11%); *Salmonella sp*. (7.26%); *Shigella sp. (6.84%); Staphylococcus aureus* (6%). Overall 76.92% isolates were resistant to ampicillin and 34.61%, 40%, 23.08% and 12.39% were resistant to third-generation cephalosporin, vancomycin, colistin and carbapenem respectively. From overall isolates, the MDR and XDR organisms were found to be 84.62% and 5.13% respectively. PCR data on ESBL and carbapenem resistance genes are ongoing.

Conclusion: High prevalence of multiple-drug-resistance bacteria in both hospital and community wastewater is indicating the dissemination of these pathogens in community which may lead to great public health issues if the situation is not tackled in near future.

Keywords: Pathogens, Antibiotic, Dhaka, Hospital wastewater, Community wastewater, Multi-drug Resistance

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List of Acronyms:

ARB	Antibiotic Resistant Bacteria
TSI	Triple Sugar Iron
MIU	Motility-Indole-Urease
MR	Methyl Red
VP	Voges-Proskauer
MHA	Mueller Hinton Agar
IMP	Imipenemase
VIM	Verona integrin-encoded metallo-β-lactamase
NDM	New Delhi metallo-β-lactamase
PCR	Polymerase Chain Reaction
AGE	Agarose Gel Electrophoresis
ESBL	Extended Spectrum Beta Lactamase

Chapter 1

Introduction

We are living in such an era where taking antibiotics for any kind of diseases or infections has become a very common scenario both in rural and urban areas. Even we take antibiotics for fever and even without consulting any doctor. But we people are skipping the fact that misuse of antibiotics and inappropriate uses of antibiotics has arisen the increasing number of antibiotic resistant bacteria as well as their resistant genes. It is very much unfortunate for the people of this generation and for every generation that the world has been introduced with a very few new effective antibiotics in the past two decades whereas it has been introduced with a great number of antibiotic resistant bacteria and antibiotic resistant genes. Though there is no time for respite and think that researchers, engineers are not trying to increase the number of effective antimicrobial drugs and are not trying to improve the effectiveness of existing antimicrobial drugs. In addition to that, almost all previously used effective antibiotics have lost their potentiality to work against infections, diseases already. They are trying their best but can't because of the loss of their activity due to bacterial resistance which leads to a substantial rise in resistant bacteria.

The excess use of antimicrobial agents as a form of antibiotics both in medical hospitals and the environment puts a selection pressure on bacteria. Since these antibiotics resistant bacteria and their responsible genes can be easily transmitted into the environment and get connected to every individual who have a strong immunity as well as who lack a strong immunity via wastewater ejaculated by hospitals and households where antibiotics are commonly and randomly being used. Bacteria that contain resistant genes against different microbial agents are not only responsible for decreasing the number of effective antibiotics but also posing a very serious public health impact because they carry transmissible genes and can be able to act as a vector or reservoir of resistant genes which may lead to another serious health issue.

However, the wastewater treatment processes can play a vital role against this problem but unfortunately except for a very few developed countries, all other countries of the world have poor wastewater treatment plants and processes. So, wastewater such as sewage that is open and untreated, drainage systems that are poorly maintained connected both with hospitals and houses are considered as hotspots for antibiotic-resistant bacteria and resistance genes.

It is high time that everyone gets alerted about this modern problem and doctors, researchers, microbiologists, clinicians, public health experts, policymakers, engineers and everyone who is related to this field can look upon this dynamic problem and can come up with some effective, extraordinary solution to cope up. Before making any decision they have to be introduced with global surveillance data. Keeping that in mind, the purpose of our study is to inform them about the prevalence and diversity of different antibiotic resistant bacteria that we have found from different well known hospitals of Dhaka city where people of various class, age, vulnerable to infections go there frequently for treatment and from different community wastewater from different areas of same city that are overloaded with residents. Furthermore, other objectives of our study is to make a comparison between pathogenic organisms found from medical wastewater and community wastewater, identify antibiotic resistant and sensitive bacteria from medical wastewater and community wastewater and compare them and Identify antibiotic resistant and sensitive bacteria from medical wastewater and community wastewater and compare them.

Chapter 2:

Background:

Uncontrolled and excessive exposure of antibiotics in the medical waste poses a selective pressure to acquire and disseminate highly antibiotic resistance genes to common pathogenic bacteria. The emergence of resistance among the most significant bacterial infections is widely acknowledged as a serious public health concern that affects people all over the world. Multidrug-resistant organisms have arisen not just in hospital environment, but also in community environment which implies that reservoirs of antibiotic-resistant bacteria exist outside of the hospital. Though the rate of antibiotic resistance is increasing day by day rapidly but people are rarely concerned about it. This study aims to determine the diversity of antibiotic resistant bacteria from hospital and community wastewater in Dhaka city.

When wastewater containing a range of harmful chemicals is released into the environment, it poses major health risks to humans, animals, and plants, while also providing a suitable setting for pathogenic and non-pathogenic bacteria to pollute the overall ecosystem. According to Kruse, H. (1999), antibiotic pollutants in hospital wastewater selectively kill or inhibit the growth of susceptible bacteria, favoring the development of resistant bacteria. As the competing flora has been wiped out by the pollution, this results in a massive surge of resistant bacteria. In literature, sewage contamination is reported as a major source of pathogenic bacteria in water resources, but various national and international authors also suggested that besides sewage contamination, the wastewaters discharged from various industries such as distillery, pulp and paper mills, pharmaceutical wastes and tannery industries etc. also act as a good source of nutrients (organic and inorganic) and support the growth of pathogenic microbes in receiving water bodies. (Lofrano et al., 2013; Wang et al., 2014). Again, pharmaceutical chemicals and resistant bacteria can easily mixed up with wastewater systems via hospital, industrial, and residential wastewater output, and ultimately be released into the environment.Hospital wastewater is a highly selective environment, according to studies, and it contributes to the high frequencies of resistant bacteria released into the natural environment. The presence of bacteriophages in samples of animal feces has been shown by Colomer-Lluch et al. to be environmental vectors for the horizontal transmission of antibiotic resistance genes. (Moges et al., 2014). According to an article of Frontiers, while the mechanisms by which antibiotic-resistant bacteria (ARB) and antibiotic-resistant Gene (ARG) are transported and spread through the environment are still unknown, previous research has suggested links between human activity and the transmission of resistance traits through agricultural operations, aquatic environments, and sediments.

According to an article of Stokes, H.W. and Gillings, M.R. (2011), the microbial fauna in hospital wastewater has developed a number of mechanisms for medication and toxic element resistance. With the help of a particular gene, most microbes evolved resistance to hazardous chemicals and antibiotics, and these genes were generally located on plasmids or chromosomes. Another report of International Journal of Microbiology demonstrated that the transfer of resistance genes between bacterial species via transduction or conjugation can potentially contribute to the rise of resistant bacteria. All of these mechanisms have the potential to create super bug/multidrug resistant bacteria that is resistant to a wide range of antibiotics. According to previous research, hospital wastewater is a significant source of drug-resistant bacteria in the environment. For example, a study done in the European region revealed that 1.5 percent of the bacteria population in hospital effluents was MDR (Multi Drug Resistant). Three multiple-resistant strains (*Acinetobacter johnsonii, Escherichia coli,* and *Citrobacter freundii*) isolated from treated hospital sewage were investigated for their ability to survive in natural waters and retain antibiotic resistance in a study conducted by the Danish environmental protection agency. Similar results were found in a Belgian study comparing the antimicrobial tolerance of tetracycline resistant heterotrophic bacteria from hospital effluent and freshwater fish farm water. According to a study done in India, the presence of MDR bacteria in hospital samples ranged from 0.26 percent to 40%, which is disturbingly high and might represent a severe health risk to the society. The prevalent MDR pattern included simultaneous resistance to ampicillin, amoxicillin-clavulinic acid, piperacillin, second and third generation cephalosporins, cotrimoxazole, gentamycin, netilmycin, and quinolones. For E. coli, Klebsiella, Enterobacter, Citrobacter, and Pseudomonas, the pattern was nearly identical, indicating the frequency of comparable R-plasmids. In addition, a study done in Gondar, Ethiopia, revealed that several drug resistant microorganisms to routinely used antibiotics are prevalent in hospital wastewater. Coliform levels were also shown to be high in hospital wastewater in previous research. (Tsegahun Asfaw, 2017)

Chapter 3:

Materials and Methods:

10 different samples were aseptically collected from different places in Dhaka city among which 5 samples were collected from Medical wastewater and the other 5 samples were collected from community wastewater for comparing the antibiotic resistant pathogenic organism counts. When collecting medical waste water, it was kept in mind that the water that comes from the medical waste pipeline, which is a raw waste, must be collected before mixing with the main sewage line. On the other hand, to compare the antibiotic resistant microbes of the hospital wastewater and community wastewater more accurately, it was necessary to collect community wastewater which does not mix with any type of medical waste and that's why it was tried to collect community samples at least 2 kilometers away from any hospital.

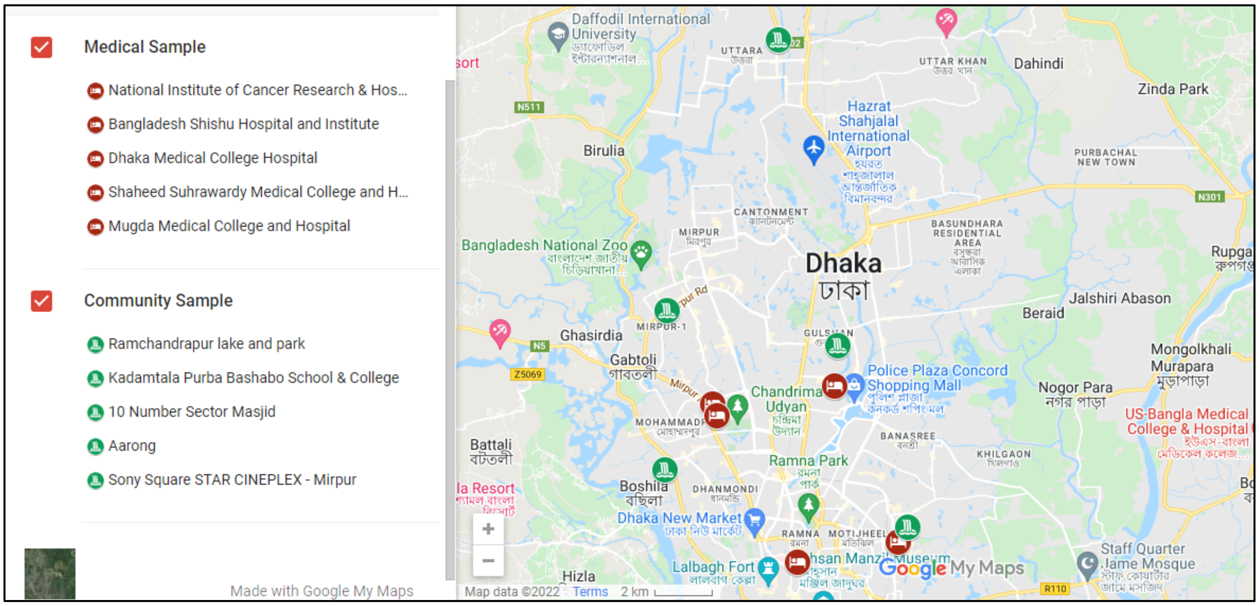
Each Sample was collected mostly early in the morning in a sterile 50 ml falcon tube at volumes between 35-40 ml and transported to the laboratory as soon as possible for further processing. For sample transportation, they were transported at 4˚C so that the composition of the sample does not disrupt in any way and also to prevent it from any kind of contamination. Then, within 4 hours they were analyzed in the laboratory.

<u>Figure 01:</u> Community Wastewater Sample Collection

Figure 02: ***Medical Wastewater Sample Collection***

From sample collection to PCR test, following 8 steps were performed sequentially:

1. 10 samples from different places in Dhaka city were collected aseptically in a 50 ml sterile falcon tube and then the sample was transported at 4 ˚C. (Figure 03: sample collection places)



☑ Medical Sample

- National Institute of Cancer Research & Hos...
- Bangladesh Shishu Hospital and Institute
- Dhaka Medical College Hospital
- Shaheed Suhrawardy Medical College and H...
- Mugda Medical College and Hospital

☑ Community Sample

- Ramchandrapur lake and park
- Kadamtala Purba Bashabo School & College
- 10 Number Sector Masjid
- Aarong
- Sony Square STAR CINEPLEX - Mirpur

Figure_03: ***10 Sample Collection Places in Dhaka City***

2. 10-fold dilution was used to culture the sample up-to 10^7 dilution on 5 five different media plates using spread plate technique. Here, Nutrient Agar Media was used for

supporting the growth of all the present microbes, Cetrimide Agar media was used to support the growth of specific Pseudomonas spp. organisms. Xylose-Lysine-Deoxycholate Media and Mac Conkey Agar Media was used to support the growth of Gram Negative organisms and Mannitol Salt Agar Media was used to support the growth of Gram Positive organisms. After 24 hours of incubation at 37˚C the plates were observed.

3. After the incubation, based on their colony morphology, the isolation was done. When same morphology observed, it was tried to choose at least one representative organism from each of them.
4. For the confirmation of the organisms, the isolated organisms from different media plates were cultured on the Hichrome UTI Agar Media and kept for incubation at 37˚C for 24 hours. After the incubation, by observing the color and texture the organisms were isolated. For example, the purple colored colony indicates the presence of *Escherichia coli*, mucoid blue color colony indicates *Klebsiella* spp., golden yellow appearance indicates the presence of *Staphylococcus spp* and so on.
5. To confirm the identification of each of the isolated colony, Gram staining and several biochemical tests were done. Gram staining is a staining process which helps to determine the identification of Gram positive or Gram negative organism. For biochemical tests, Oxidase, Catalase, Triple Sugar Iron Agar Test (TSI Test), Methyl Red (MR) Test, Voges-Prouskauer (VP) Test, Citrate Utilization Test, Motility-Indole-Urease (MIU) test were performed for the each isolated colony. Here we took help from ABIS Bacterial Identification software for confirming the identification of the organisms. Again, we have compared with the biochemical result reference chart (Given below) for more accuracy.

Organism	Gram stain	Agar slant culture characteristics	Fermentation				NO$_3$ reduction	Indole production	MR reaction	VP reaction	Citrate use	Urease activity	Catalase activity	Oxidase activity	Gelatine liquefaction	Starch hydrolysis	Lipid hydrolysis
			Lactose	dextrose	sucrose	H$_2$S											
E. coli	Rod (-)	White, moist, glistening growth	AG	AG	A +/-	-	+	+	+	-	-	-	+	-	-	-	-
Enterobacter aeruginosa	Rod (-)	Abundant, white, thick, glistening growth	AG	AG	AG +/-	-	+	-	-	+	+	-	+	-	-	-	-
K pneumoniae	Rod (-)	Slimy, white, somewhat translucent growth	AG	AG	AG	-	+	-	-	+/-	+	+	+	-	-	-	-
Shigella dysenteriae	Rod (-)	Thin, even, grayish growth	-	A	A +/-	-	+	+	+	-	-	-	+	-	-	-	-
S typhimurium	Rod (-)	Thin, even, grayish growth	-	AG +/-	A +/-	+	+	-	+	-	+	-	+	-	-	-	-

P vulgaris	Rod (-)	Thin, blue, gray, spreading growth	-	AG	AG +/-	+	+	+	+	-	-	+	+	-	+	-	-
P aeroginosa	Rod (-)	Abundant, thin, white growth with medium turning green	-	-	-	-	+	-	-	-	+	-	+	+	+ rapid	-	+
A faecalis	Rod (-)	Thin, white spreading viscous growth	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
V cholerae	Comma shaped (-)		-	A-	A -	?	+	+	?			-	?	+	+	+	+

***<u>Table 01:</u>* Biochemical Reference Chart**

6. As the aim of the research was to compare the antibiotic resistance pattern for the organisms between medical and community wastewater, we have performed the antibiotic susceptibility testing. It was tried to use antibiotic disks from every group of antibiotics. For instance, from Penicillin group Ampicillin (AMP-10 mg); from Fluoroquinolone group Ciprofloxacin (CIP-5 mg); from Macrolides group Azythromycin (AZM-15 mg); from Glycopeptides group Vancomycin (VA-30 mg); from Tetracycline group Tetracycline (TE-30 mg); from Cephalosporin group Ceftriaxone (CTR-30 mg) and Cefuroxime (CXM-30 mg); from Carbapenem group Imipenem (IMI-10 mg); from Aminoglycoside group Amikacin (AK-30 mg); Chloramphenicol and Colistin sulphate. The organisms were allowed to grow on the Mueller-Hinton Agar (MHA) Media and

then after placing the disks maintaining a proper distance according to their zone of inhibition, the plates were observed after 24 hours of incubation at 37˚C. According to the Clinical Laboratory Standard Institute's (CLSI) guideline, the zone were measured and identified as Sensitive, Intermediate or Resistant.

7. After identifying the resistant organisms for particular Carbapenem , Penicillin, Cephalosporin and Colistin sulphate group of antibiotics, the organisms were chosen for DNA extraction to identify the responsible gene for the resistance pattern of the organisms. Here to extract the bacterial DNA, boiling method was used where extreme heat (95˚C) is given to the bacterial suspension after addition of the nuclease free water. By rupturing the bacterial cells, the DNA comes out and remains in the nuclease free water.
8. To perform the PCR test, particular primers for the specific gene were taken. For instance, To identify carbapenem resistance gene, "bla NDM" and "bla IMP"; for Beta Lactamase gene for ESBL resistant, "bla CTX", "bla TEM", "bla OXA" and "bla VIM" and for Colistin Sulphate resistance gene, "CLR" has been used. The extracted DNA of the target organisms along with the specific primers were mixed and prepared a 25 µl of master mix to run the PCR. Then Agarose Gel Electrophoresis (AGE) was performed to identify the presence of the specific gene by observing the DNA band size.

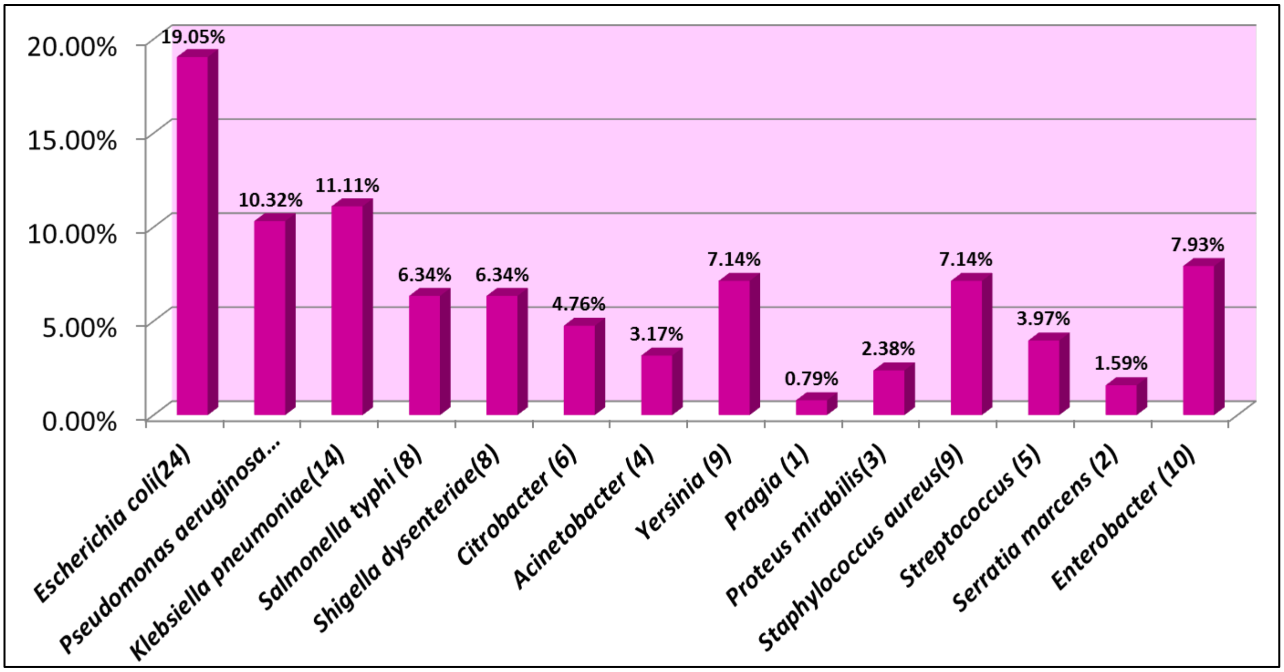
Chapter 4

Result

There are a total of 234 isolates on different selective media in this study. Medical Wastewater had a percentage and number of isolates of 46.15% (108 out of 234), whereas Community Sewage had a percentage and number of isolates of 53.85& (126 out of 234). In Medical wastewater samples 86.11% isolates are Gram negative and 13.88% samples are Gram positive. Furthermore, we found that 84.13% of community sewage is gram negative and 15.87% is Gram positive. So, in comparison to community sewage, we can clearly say that Gram negative isolates in medical waste water samples are abundant.

Name of the Organism	**Medical**	**Community**
E.coli	10.68%	19.05%
Klebsiella spp.	15.53%	11.11%
Pseudomonas aeruginosa	5.83%	10.32%
Salmonella spp.	8.74%	6.34%
Shigella spp.	7.77%	6.34%
Enterobacter spp.	10.68%	7.93%
Staphylococcus spp.	4.85%	7.14%

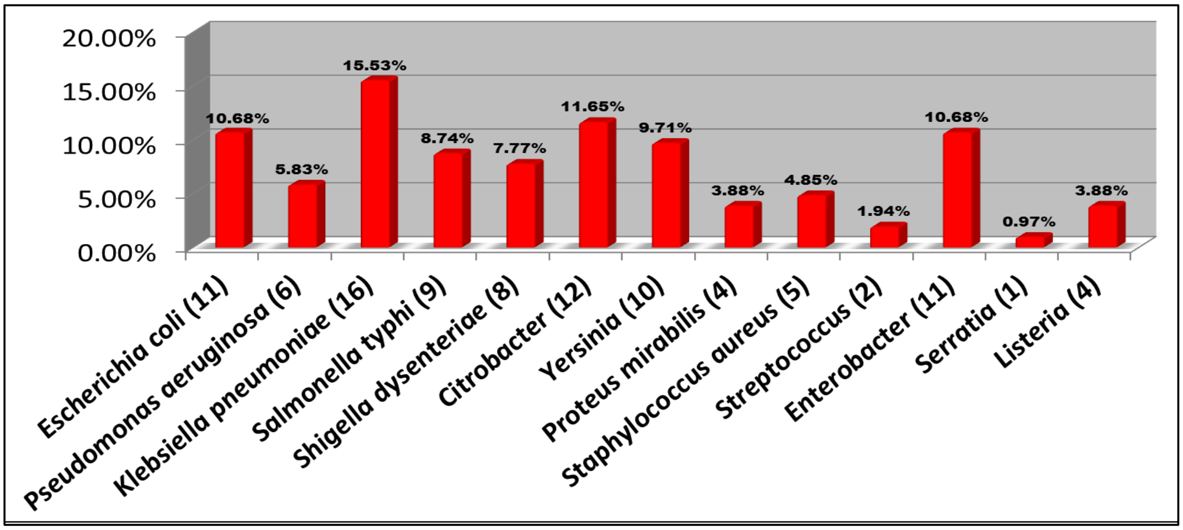
***Table: 02:* Organism Load in Medical and Community Wastewater**



<u>Figure: 04:</u> Percentage of Organisms from Community Wastewater

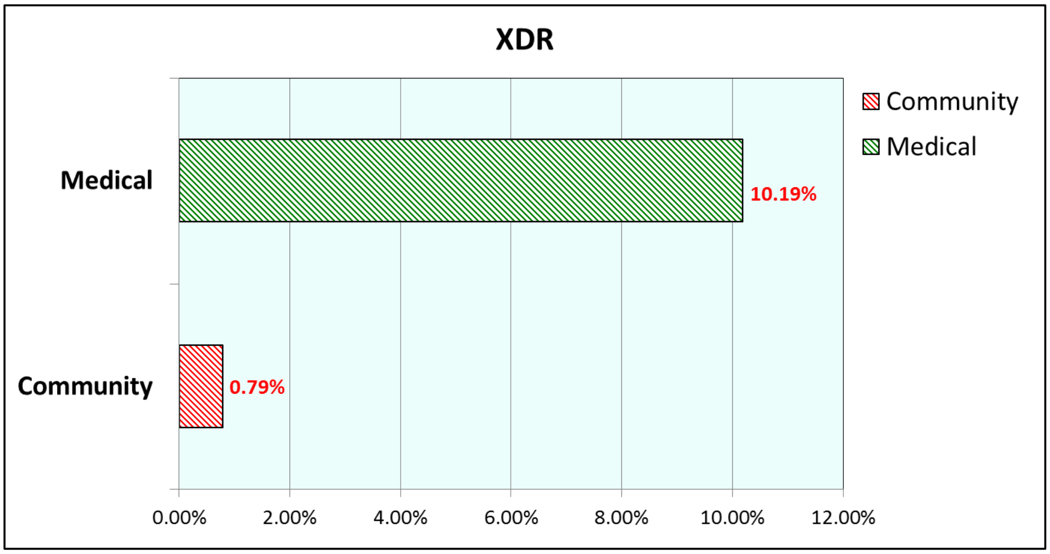
In community sewage, Gram Negative isolates included 19.05% *E.coli*, 11.11% *Klebsiella spp, 10.32% Pseudomonas spp, 6.34% Salmonella spp.* as well as S*higella spp.* and *7.93% Enterobacter* while Gram positive isolates included *7.14% Staphylococcus spp.* as well.

On the other hand, in Medical Waste water, Gram Negative isolates included 10.68% *E.coli, 15.53%, Klebsiella spp, 5.83% Pseudomonas spp., 8.74% Salmonella spp., 7.77%* Shigella *spp.* and *10.68% Enterobacter* while gram positive isolates included *4.85% Staphylococcus spp.* as well.



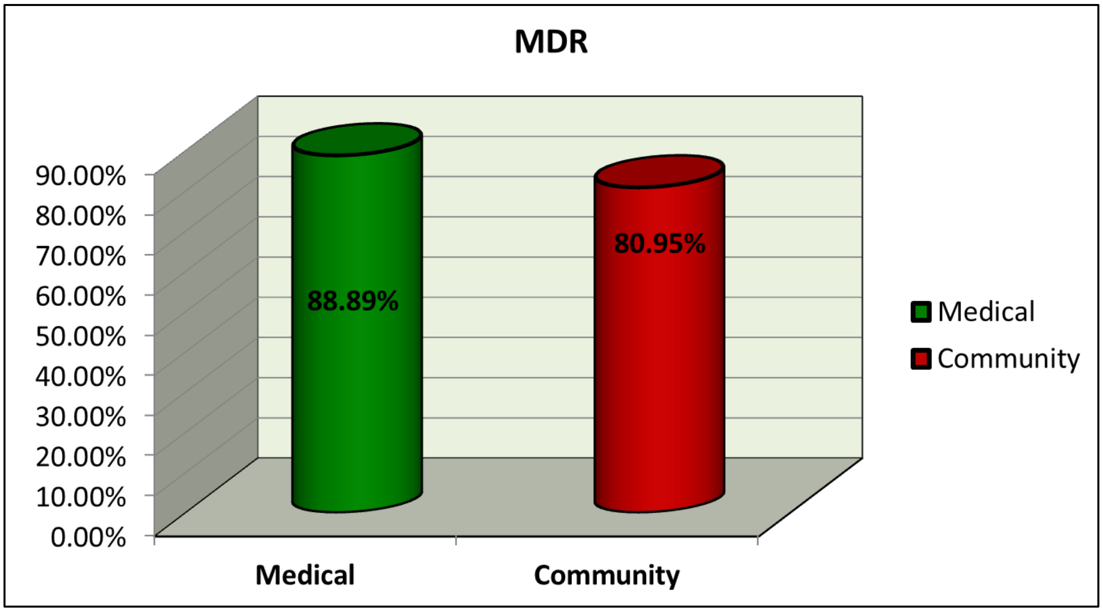
<u>Figure:05:</u> Percentage of Organisms from Medical Wastewater

Antimicrobial assay results



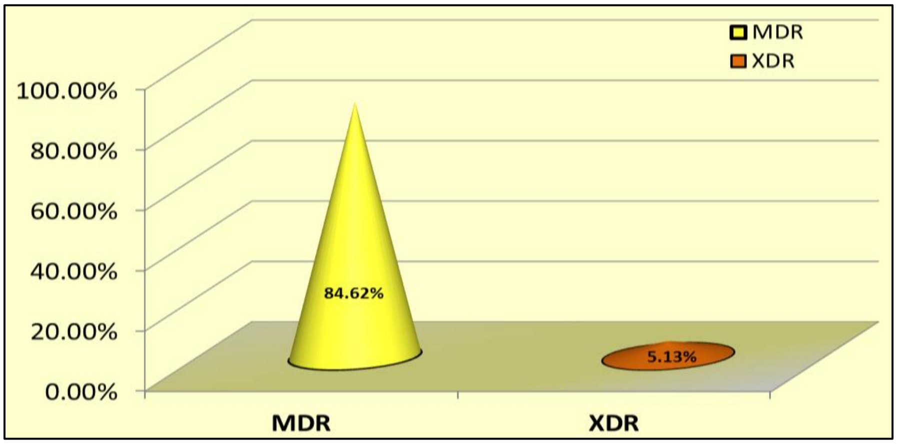
<u>Figure: 06:</u> XDR Organism Load in Medical vs. Community Wastewater

If we look at the load of XDR (Extended Drug Resistant) organisms in community sewage and medical waste, we can see that there is a significant difference. Overall from medical waste water isolates 10.19% were found to be defined as XDR, on the other hand, only 0.79% isolates were found to be resistant from community sample. Here, the XDR organisms were identified by considering their sensitivity to less than 3 groups of antibiotics from the 10 different groups of antibiotics that were tested. So, here the XDR load is significantly higher in medical wastewater than community sewage sample.



<u>Figure 07:</u> MDR Organism Load in Medical vs. Community Wastewater

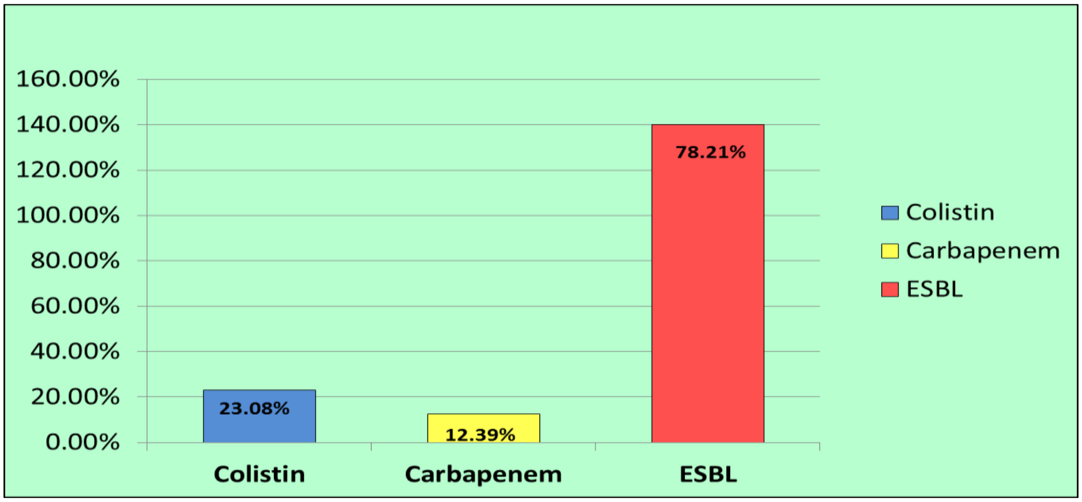
The above mentioned graph shows about the MDR load variation between Medical and Community Wastewater. Here, the MDR load variation between community sewage and medical waste water is also noticeable. It is clearly shown in the graph that the MDR load is higher in medical waste water which is 88.89% whereas in community it is found to be 80.95%. Here, the organisms were defined as MDR or Multi Drug Resistant which were found to be resistant for more than 2 groups of antibiotic in between the 10 groups of antibiotic used here. Though the variation in percentage is not significant but then again the MDR load is higher in medical wastewater than community wastewater.



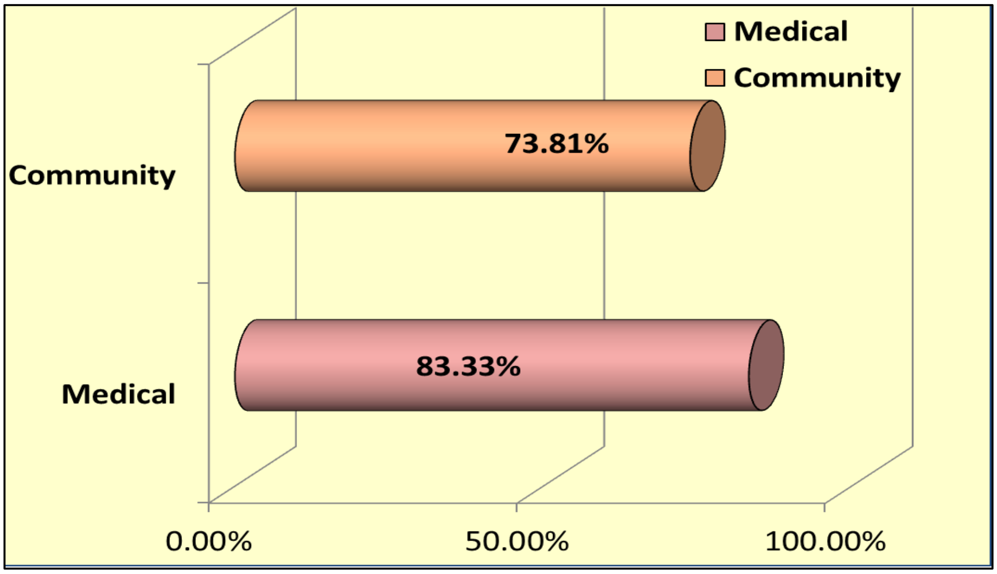
<u>Figure 08:</u> Comparison between MDR and XDR Load

The graph here shows the overall comparison in between XDR and MDR. So, from the total isolates 84.62% or nearly 85% organisms were found to be resistant to more than 2 groups of antibiotics. On the other hand, only 5.13% organisms showed resistance pattern as XDR organisms among all the isolates. So, this data indicates that MDR load is significantly higher than XDR organism.

The following graph shows the Colistin, Carbapenem and ESBL resistant organisms from the overall 238 isolates. Here the most common resistant pattern is shown by ESBL producing organisms which are 78.21%. The second highest percentage is carried by Colistin resistant organisms which are 23.08% and carbapenem resistant organisms hold 12.39%. So, it is clear that most of the organisms are highly resistant to ESBL.

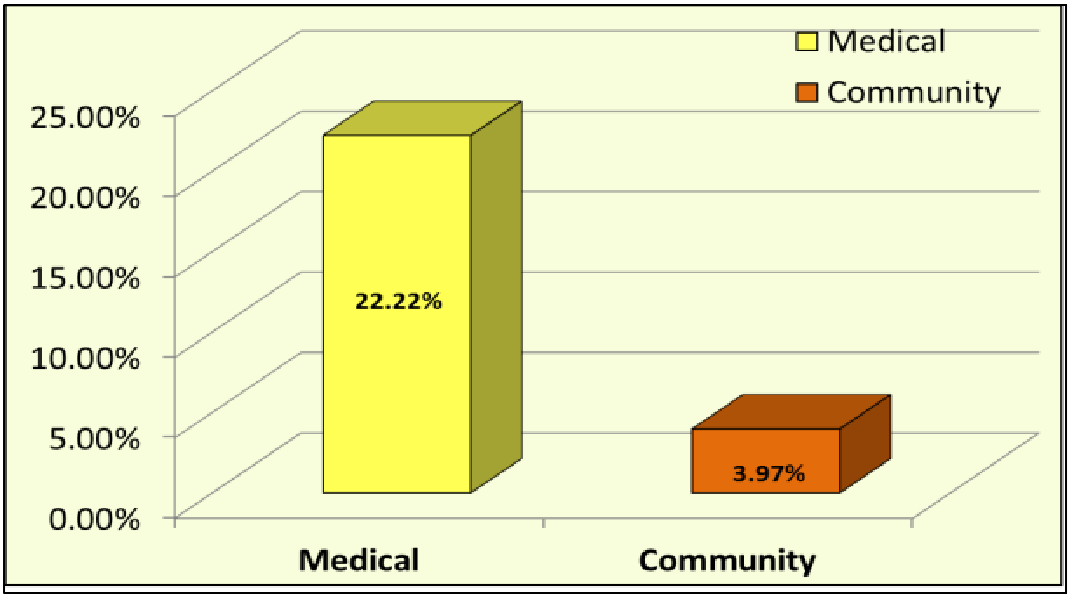


<u>Figure 09:</u> Colistin vs. Carbapenem vs. ESBL Resistance pattern



<u>Figure: 10:</u> Comparison of ESBL producing organism between the medical and community Wastewater

We can observe that the Extended Spectrum Beta Lactamase or ESBL producing organism's comparison between medical and community wastewater samples from the graph. Here the resistance pattern is high in medical waste and that is 83.33%. On the other hand, in community samples, the resistant pattern for ESBL producing organism was found to be nearly 74%. The organisms that showed resistance to Penicillin and Cefalosporin group of antibiotics those were indicated as ESBL producing organisms.



<u>Figure: 11:</u> Comparison of Carbapenem resistant organism between the medical and community Wastewater

This graphical representation indicates the comparison of carbapenem resistant organism between the medical and community Wastewater. Here, we observe that 24 isolates out of 108 isolates from medical waste water and 5 isolates from 126 community sewage were found to be resistant to the carbapenem group of antibiotic. So, the percentage was found to be 22.22% for medical waste water which is much higher than community sewage which was found to be nearly 4%.

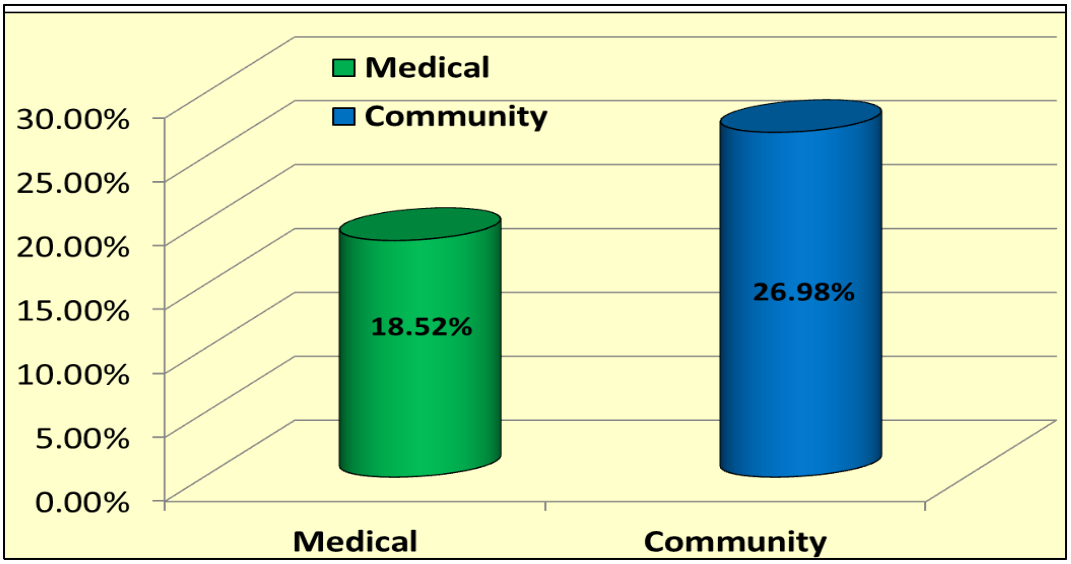


Figure: 12: ***Comparison of Colistin sulphate resistant organism between the medical and community Wastewater***

The graph represents the resistance pattern for Colistin Sulphate. Moreover, we can see that Colistin resistance is higher in community sewage which is 26.98% than medical waste water which is 18.52%. Usually, the medical waste water shows a higher resistance pattern but this data is quite opposite here, the community sewage sample showed a higher resistance pattern for colistin than the medical waste water samples.

If we see the comparison between the Vancomycin resistant *Staphylococcus spp* in between medical and community waste water. From the following graph, we can see that 50% of medical isolates were found to be resistant to Vancomycin. From the Community Sewage sample 33% of them were found resistant to Vancomycin. Overall, 40% *Staphylococcus spp* were resistant to Vancomycin.

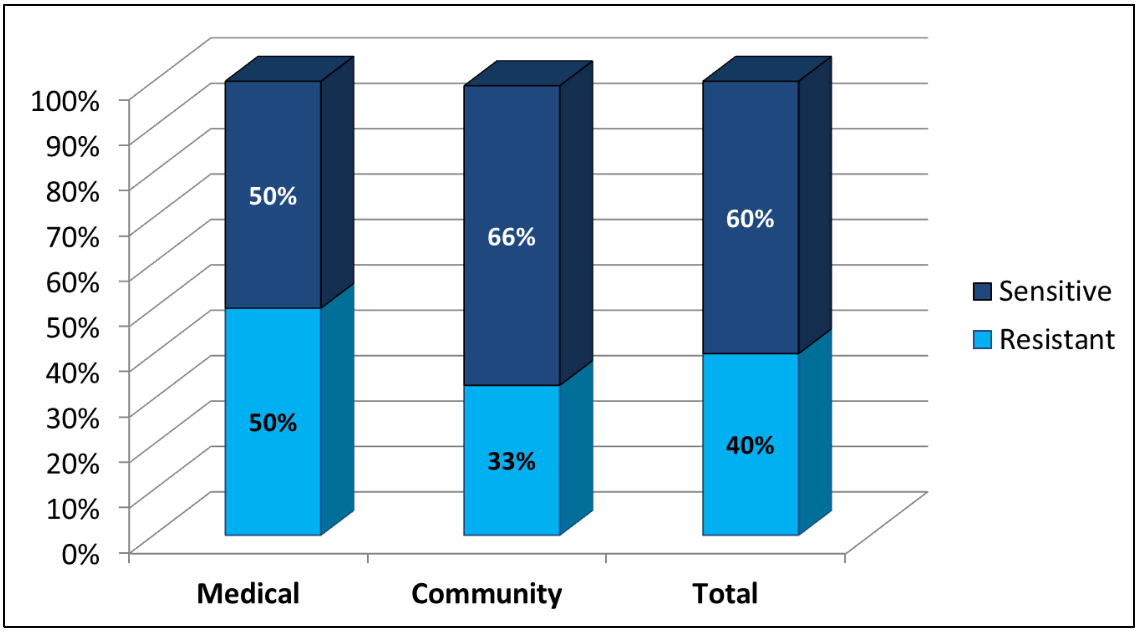


Figure 13: ***The comparison between the Vancomycin Resistant Staphylococcus spp. in between Medical and Community Wastewater***

After observing all the data, we can conclude that the count for organisms were higher in community wastewater than medical wastewater. On the other hand, in case of antibiotic resistance pattern, medical waste water showed more resistance than community wastewater.

Antibiotic	**Medical**	**Community**
AMP	84.28%	78.22%
CIP	30.24%	30.32%
AZM	63.72%	42.13%
MRP/IMP	25.92%	6.30%
CT	21.60%	42.84%

Table: 03: ***Percentage of Isolates Resistance to Antibiotics***

Chapter 5

Discussion:

The number of bacterial colony isolation from the medical and sewage wastewater samples were different and among 234 different isolates, 108 isolates were from medical samples whereas 126 isolates were from community wastewater. Not only were the numbers but also there found some notable differences between these two types of sample. The probable reason behind the number of pathogenic organisms is higher in community wastewater than medical samples could be in medical due to treatment purposes different type of antimicrobial agents are being used continuously and as a result cell lysis of bacteria, degradation in cell components, creating a stress in bacteria, losing the activity participate in the process of decreasing CFU. But community wastewater lacks such antimicrobial agent density and the number of CFU remains high. Furthermore, the ratio of Gram-negative bacteria in medical samples was higher (86.11%) than that of the community wastewater (84.13%). On the other hand, the ratio of Gram-positive bacteria in community wastewater samples was higher (15.87%) than that of the medical samples (13.88%). Gram negative bacteria are highly responsible for urinary tract infections. Also, the thinner layers of peptidoglycan of gram positive bacteria are responsible for their strong survival rate. Although the number of bacterial isolates was higher in community wastewater than that of medical samples, both the XDR and MDR organisms were found higher in number from medical samples. The probable reason behind the higher number of XDR and MDR organism in medical samples than community wastewater could be as hospitals use high number of antimicrobial drugs, there is much possible ways for bacteria to become itself resistant to these agents and develop required resistant genes against these agents whereas community wastewater lack of these agents and these ways to become resistant itself. In addition, the highest percentage of pathogenic organism that were resistant to different antimicrobial agents that had been found from community wastewater samples was for *E. coli* which was 19.05% whereas the highest percentage of pathogenic organism that were resistant to different antimicrobial agents that had been found from medical samples was for *klebsiella* *pneumonia* which was 15.83%. *E. coli* is responsible for mostly and frequently occurring infections such as UTIs, bacteremia, cholangitis, meningitis and so on. *Klebsiella pneumonia* is responsible for UTIs, pneumonia, meningitis, intra-abdominal infection and so on. Again, when we compared the total count of predominant organisms we had found that except for *pseudomonas* and *staphylococcus* almost all other pathogenic bacterial counts were higher in community wastewater samples which is a threat to human health. Furthermore, we had isolated *Acinetobacter* from community wastewater which we didn't find from medical samples. In contrast, we had isolated *Listeria* from medical samples but didn't find any from community wastewater. The lower number of organisms that we found on community wastewater is for *Pragia* (0.79%) whereas the lower number of organisms that we found on medical samples is for *Serratia* (0.97%). To compare some positive organisms that we found we had isolated *Staphylococcus aureus, Streptococcus, Enterococcus, Bacillus cereus, Listeria monocytogenes* etc. Among of these gram positive organism in community wastewater *Staphylococcus aureus* (7.14%) was found higher in number whereas in medical samples *Staphylococcus aureus* (4.85%) was also found higher in number though in both type of samples the ratio is significantly different from each other.

We performed antibiotic susceptibility tests in order to observe the antimicrobial susceptibility pattern of different isolates that we had found from all of our samples. In our study we found that the most common resistant pattern is shown by ESBL producing organisms (78.21%.). It is known that Extended Spectrum Beta Lactamase is an enzyme produced by bacteria which makes the bacterial infection harder to treat with antibiotics like penicillin and cephalosporin group. Again, the second highest percentage was carried by colistin resistant organisms (23.08%). Colistin sulphate is a specific antibiotic that is used to treat infections caused by Gram negative Multi Drug Resistant organisms. Lastly, the next percentage was found for carbapenem resistant organisms (12.39%). When we compared these percentages of our result we saw that most of the organisms are highly resistant to ESBL both in medical samples (83.33%) and in community wastewater (83.33%). Furthermore, for the group of carbapenem antibiotics, the percentage was found to be 22.22% for medical waste water which is much higher than community sewage which was found to be nearly 4%. In addition, colistin in the medical waste water shows a higher resistance pattern but we found quite the opposite result of that. Our study showed the community sewage sample contained a higher resistance pattern for colistin than the medical waste water samples. When we particularly observed the data of gram positive organisms for antibiotic susceptibility test we saw overall, 40% Staphylococcus spp were resistant to Vancomycin. It is known that vancomycin has been the most reliable therapeutic agent against infection caused by Methicillin Resistant *Staphylococcus spp*. To sum it up, it is clear to us that between community wastewater and medical samples though the number of isolates had been found more than that of medical samples, we found more MDR and XDR organisms from medical samples.

The presence and percentages of both MDR and XDR organism both in medical samples and community samples indicate the probability of how fast the opportunistic organisms that are commonly found in the environment and are not as pathogenic as they can harm human health are going to be resistant with the help of already become resistant organism and their resistant genes. Not the people who are not concerned about the threat but also those who are well known about the facts and future are being affected by this problem at the same rate.

Chapter 06

Conclusion:

Though we tried our level best to perform our study yet we had some limitations. In this study isolations of bacterial colony had been performed based on morphology and size but it is known to us that a same species of bacteria can grow in different medias, such as *E. coli* which can grow in NA media, MacConkey agar media, XLD agar media, Cetrimide agar media so it was not possible for us to separate same species and we had picked up *E. coli* from more than one media and it consumed our time, resources, energy. Also, due to lack of time and resources we weren't able to collect more than 10 samples outside of Dhaka city.

To sum our study up, we would like to emphasize on the fact that, in order to survive the present and future situation that is going to arise due to antibiotic resistant organisms and antibiotic resistant genes proper wastewater treatment plants should be enforced and implemented both in medical sewages and community sewages. In addition, every medical professional should follow all the rules set by the government properly to cope up with the situation and if they don’t follow these rules strictly they should be punished. Not only the government and medical but also we general people who are the main reason behind the increasing number of XDR and MDR organisms as we take unnecessary, randomly, more than required antibiotics, we should be concerned about it.

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