DOI: https://doi.org/10.53350/pjmhs20221611459

ORIGINAL ARTICLE

Prevalence and Antimicrobial Susceptibility Pattern of Enterobacteriaceae Isolated from Hospitals Wastewater

MUHAMMAD RIZWAN[1], HABIB ULLAH[2], ANJALA AYOUB[3], HARIS ABDUL REHMAN[4], MUHAMMAD UMAR*[5], AQSA QURBAN[6], MUHAMMAD ASLAM[7], USAMA[2], Maria Kausar[8], AAMNA SYED[6]
[1]*Department of Microbiology, Government College University, Faisalabad, Pakistan*
[2]*Anhalt University of Applied Sciences, Anhalt, Germany*
[3]*Department of Biotechnology, University of Gujrat, Gujrat, Pakistan*
[4]*Department of Microbiology, University of Central Punjab, Lahore, Pakistan*
[5]*Department of Biochemistry, Government College Women University Faisalabad, Pakistan*
[6]*Department of Life Sciences, University of Management and Technology, Lahore, Pakistan*
[7]*Department of Microbiology, Quaid e Azam University, Islamabad, Pakistan*
[8]*Department of Parasitology, University of Agriculture Faisalabad, Pakistan*
Corresponding author: Muhammad Umar; Email: muhammadumar@gcwuf.edu.pk, Cell: 0335-7755516

ABSTRACT

Background: Hospital wastewater (HWW) is bearing in mind the main root to proliferate multidrug-resistant among the Enterobacteriaceae family. Enterobacteriaceae is suited to be a considerable risk for antibiotic-resistant through CTX-M production which is observed as an important factor leading to multiple drug-resistant.

Study Design: A total of 100 samples of HWW and community wastewater were collected aseptically. Samples were sub-cultured through basic, selective, and UTI Chrome agar.

Place of the study: This research was conducted in the Postgraduate Research Laboratory of the Department of Microbiology, Government College University Faisalabad.

Duration of the study: The duration of the study was started from September 2018 to May 2019.

Methodology: Bacteria were identified based on cultural characteristics and different biochemical tests. Antimicrobial susceptibility testing was performed as per CLSI 2016 guidelines. The molecular characterization of the CTX-M was done bt PCR to present its association with multidrug-resistant.

Results: Among the total 165 isolates, the predominant bacteria were E.coli (48.1%) followed by K. pneumonia (25.3%), Shigella species (8.0%), and P.aeruginosa (12.7%) from hospital wastewater while E.coli (30.3%) followed by K. pneumonia (32.6%), Shigella species (20.9%) and P.aeruginosa (11.6%) were isolated from community wastewater. The AST of CTX-M producers and Non-CTX-M producers was also compared to find the relation of CTX-M to multidrug-resistant. Tigecycline was observed as a predominant resistant (≥96%) antibiotic followed by meropenem (≥40%) against all isolated CTX-M producers.

Conclusion: The conclusion represented the existence of MDR Enterobacteriaceae in untreated hospital wastewater. All efforts revealed realize the requisite that hospital wastewater should be treated with standard treatment plants before exposing it to community wastewater.

Keywords: Hospital wastewater; Antibiotic Resistance genes; CTX-M producers; Urinary Tract Infection (UTI),Enterobacteriaceae

INTRODUCTION

Hospital wastewater (HWW) has become a key source of antimicrobial resistance (AMR) in multiple organisms, especially in Gram-negative bacteria.In HWW, the existence of an intensive rate of MDR bacteria may be a root to enhance distribution and proliferation of ARGs through the conjugation of DNA elements of bacteria like integrons, transposons, and plasmids (Stalder et al., 2014).Laterally, these wastes water should be treated by the sewage treatment plant (STP) and then wind up in the environment, primarily in the water compartments (Pauwels & Verstraete, 2006).The existence of antimicrobial agents in hospital water waste has led to intensive environmental risk for plants and animals present in the aquatic environment. Other foundations may comprise the removal of fallow antibiotics and the disposal of waste by pharmaceutical industries (Peleg & Hooper, 2010). Evermore enteric bacteria were being investigated in hospital wastewater intheUSA, In which antibiotic resistance (AMR) caused infections in 2 million patients during stayed in hospitals and led to a minimum of 23,000 death every year (Ventola, 2015).The antimicrobial selective pressure enhanced the transmission of resistance genes among intra and inters species. According to French data from 2011, about 3 to 5% of extended-spectrum β lactamases (ESBL) entero-bacteria were accountable for urinary tract infection (UTI), and approximately 5% generated asymptomatic carriers of extended-spectrum β lactamases (ESBLs) in children as well as adults (Hocquet, Muller, & Bertrand, 2016). The rate of ESBLs was more proliferated through hospital wastewater due to the large usage of antibiotics and disinfectants (Hocquet et al., 2016).

Subjected bacteria mainly Gram-negative bacteria cause mutation which mostly leads to antibiotic resistance (Ochman, Lawrence, & Groisman, 2000). In our study, Enterobacteriaceaespecies were the main landscapes. The members of this family are vastly effective for up-regulating or causing a mutative mechanism of antimicrobial resistance. It may be due to the availability of multiple mechanisms against the same antibiotic or a single mechanism against multiple antibiotics (Akter et al., 2012). Due to this, drug resistance is the main threat to enhancing the discovery and development of new antibiotics (Boucher et al., 2009). Naturally, genes transfer horizontally by conjugation mechanism in the technical system when these bacteria come too close to each other (Barkay, Kroer, Rasmussen, & Sørensen, 1995). Enterobacteriaceae were isolated from almost all wastewater of hospitals. As a consequence of this, a perfect snowstorm has been produced with the existence of these infections and causes intensive resistance in the absence of newly developed drugs. Mostly Gram-negative bacteria cause hospital-acquired infection, out of which, Enterobacteriaceae was most noted (Peleg & Hooper, 2010).

Concerning the Centers for Disease Control and Prevention (CDC) reports, about 220 tons of antibiotics were manufactured every year in only the United States. Out of which, 50 percent is dispensed by humans(Butaye, Devriese, & Haesebrouck, 2003; Diab, Al-Turk, Ibrahim, & Al-Zhrany, 2008). The increasing antibiotic resistance squashed the efficiency of other antibiotics available at that time(Liu, Yao, Chapman, Su, & Wang, 2020). The preventive measures were to compact the extension of antimicrobial resistance and adopt strategies to reduce MDR (Canton & Morosini, 2011; Davies & Davies, 2010).

Nosocomial infections(Mosadeghrad, Afshari, & Isfahani, 2021) were a foremost encounter for patient care. It was projected that in 2002, about 1.7 million people were infected by nosocomial

infections and 99,000 patients expired associated with nosocomial infections (Klevens et al., 2007). It was the leading sixth cause of death in the United state (Kung, Hoyert, Xu, & Murphy, 2008) and the comparable report had subjected from Europe (Chopra et al., 2008). It was appraised that the US health modest was $5 billion to $10 billion expensed annually (Stone, Hedblom, Murphy, & Miller, 2005). But typically third or more nosocomial infections were avertable (Dubberke, 2014; Yokoe et al., 2014).

Microorganisms such as Gram-negative rods including Enterobacteriaceae and other non-lactose fermenters such as Pseudomonas aeruginosa existed during human infection for long period in the environment (Bradford, 2001; Xu, Zhou, Goldstein, & Jin, 2005). Most importantly strains of Gram-negative bacteria were noted from treated and untreated hospital effluents. About 58 % of isolated samples had as a minimum one antibiotic resistance and 25% had three or more antibiotics resistance such as penicillin, sulfamethoxazole, trimethoprim,lincomycin, ofloxacin and ciprofloxacin (Butaye et al., 2003; Diab et al., 2008).The main aim of this study was to formulate strategies to compact antimicrobial resistance through hospital wastewater-like Stewardship programs. This study was designed to determine the prevalence of antimicrobial resistance in Enterobacteriaceaeisolated from HWW.

METHODOLOGY

Study setting: This research was conducted in the Postgraduate Research Laboratory of theDepartment of Microbiology, Government College University Faisalabad. Allinformation regarding the numbers of the sample as per the location and site of hospitalsaround the region of Faisalabad was recorded.

Study design: It was an observational cross sectional study.

Duration of the study: The duration of the study was started from September 2018 to May 2019.

Collections and transportation of Hospital Wastewater: A total of 100 wastewater samples were collected from Private and Governmenthospitals including different canals of Faisalabad. In hospitals, different sites wereselected for sampling like drainage outside of different wards while for community wastesamples, different locations were selected including canals in the region of Faisalabad.All samples were collected from each site by using standard aseptic techniques in clean,dry, leak-proof 20ml screw-cap containers. All samples were transported to the laboratorywithin 1-2 hours in a transporting ice pack box having 2-8°C temperature.

Inclusion selection: Only Enterobacteriaceae (Gram-negative bacteria) were included in this studyand Gram-positive bacteria were excluded.

Quality assurance: All samples were collected in sterile screw-cap containers by using asepticteniques and followed personal protective equipment. All the media were usedaccording to their standard operating procedures and all quality controls were testedbefore use. Antimicrobial susceptibility testing of Enterobacteriaceae was done throughthe guidelines of CLSI 2016. All antibiotic disks were placed in a refrigerator having atemperature range from -10°C to – 20°C after using. Expired discs could not be used anddiscarded.The methodology of this study is involved in the steps that have been shown in the [figure 01].

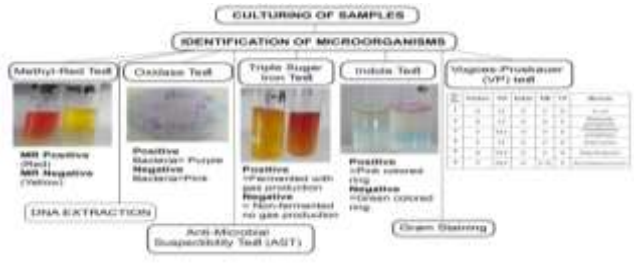

Figure 01: Flowchart of research design prevalence and antimicrobial susceptibility pattern of entrobacteriaceae isolated from HWW

RESULTS

Out of 100 wastewater samples, 165Gram-negative bacteria were isolated. A total of 79 Gram-negative bacteria were isolated from different hospitals in Faisalabad while 86 Gram-negative bacteria were isolated from different canals and waste drainages around hospital areas (Figure 02). Among these, the predominant bacteria were E.coli(n=64) followed by K. pneumonia (n=48), Shigella species (n=25), and Pseudomonas aeruginosa (n=20). The less common bacteria were Serratiamarcescens (n=5) and Salmonella species (n=3). Out of 79 isolated Gram-negative bacteria from Hospital Wastewater, 48.1% were E.coli followed by 25.3% K. pneumonia, 12.7% Pseudomonas aeruginous, and 8.0% Shigella species. Out of 86 isolates of Gram-negative bacteria from Community Wastewater, 30.3% were E.coli followed by 32.6% K. pneumonia, 11.6% Pseudomonas aeruginous, and 20.9 %Shigella species (Table 01).

Tables 02 and 03 showed the percentage of antimicrobial resistance of Gram-negative bacteria isolated from hospital wastewater and community wastewater that were sensitized against different antibiotics. All isolatedE.colifrom hospital wastewater were 100% resistant against cefepime and ceftriaxone. Other resistant patterns of E.coli against other antibiotics were tigecycline (94.73%), ciprofloxacin (73.68%), and ampicillin-sulbactam (71.05%) noted while less resistant against polymyxin B (2.14%), colistinsulfate (10.52%) and amikacin (26.31%) noted (Table 02). All isolated K. pneumonia from hospital wastewater were 100% resistant against tigecycline while other resistant patterns against these bacteria were meropenem (95.00%) and trimethoprim-sulfamethoxazole (55.34%) noted. The less resistant patterns against these bacteria were colistinsulfate (5.03%), amikacin (5.09%), polymyxin B (5.14%) and ceftriaxone (10.48%) noted. All isolatedShigella species from hospital wastewater were 71.42% resistant against tigecycline while 42.85% resistant against ciprofloxacin and ampicillin sulbactam. The less resistant patterns of these bacteria were 14.28% against cefepime, ampicillin-sulbactam, ceftriaxone, colistinsulfate, meropenem, amikacin, trimethoprim-sulfamethoxazole, and polymyxin B. All isolated Pseudomonas aeruginosafrom hospital wastewater were 100% resistant against tigecycline while others were ciprofloxacin (50%) and ampicillin-sulbactam (40.0%) noted. The less resistant patterns of these bacteria were 20.0% against amikacin, trimethoprim-sulfamethoxazole and piperacillin-tazobactam noted while 10.0% resistant against cefepime, ceftriaxone, colistinsulfate, meropenem, tigecycline, and polymyxin B noted (Table 02).

All isolated E.coli from community wastewater were 100% resistant to cefepime. Other resistant patterns of E.coli against other antibiotics were tigecycline (94.73%), ceftriaxone (80.76%) and ciprofloxacin (76.92%) noted while less resistant 3.84% against polymyxin B, amikacin and colistinsulfate (7.67%) were noted (Table 03). All isolated K. pneumonia from community wastewater was 100% resistant against tigecycline while other resistant patterns against these bacteria were low. The less resistant patterns against these bacteria were 3.57% against cefepime, polymyxin B, 7.14% against amikacin and colistinsulfate while 28.57% against piperacillin-tazobactam and ciprofloxacin noted. All isolatedShigella species from community wastewater were 100% resistant against tigecycline while 77.78% resistant against ampicillin-sulbactam and 44.45% against piperacillin-tazobactam. The less resistant patterns of these bacteria were 5.55% against ceftriaxone, cefepime, amikacin, polymyxin B, and colistinsulfate while trimethoprim-sulfamethoxazole (11.12%), meropenem (33.34%), and ciprofloxacin (38.88%) noted. All isolated Pseudomonas aeruginosafrom community wastewater were 80% resistant against ampicillin-sulbactam, and tigecycline while 60% against piperacillin-tazobactam. The less resistant patterns of these bacteria were 10.0% resistance against ceftriaxone, cefepime, trimethoprim-sulfamethoxazole, ciprofloxacin, meropenem, polymyxin B and colistinsulfate noted (Table 03).

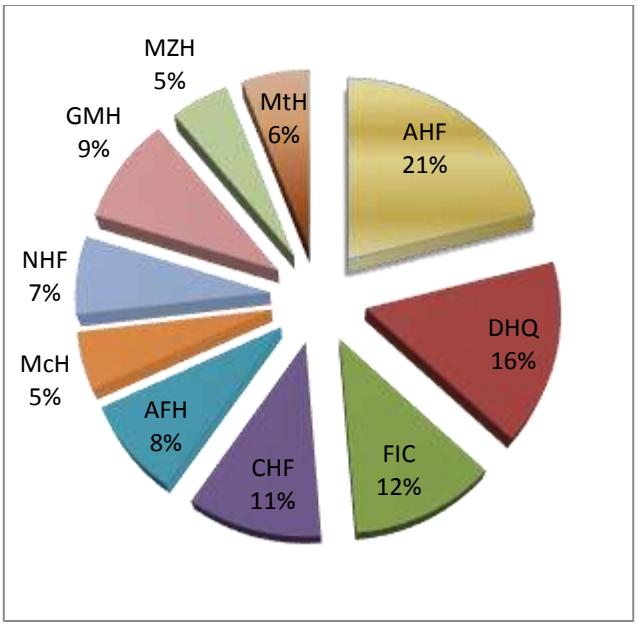

Figure 02: Percentage of Gram-negative bacteria isolated (n=79) from hospitals of the Faisalabad region.Abbreviations; Allied Hospital Faisalabad (AHF), District Head Quarter Faisalabad (DHQ), Faisalabad Institute of Cardiology (FIC), Children Hospital Faisalabad (CHF), Aziz Fatima Hospital (AFH), Manchester Hospital (McH), National Hospital Faisalabad (NHF), Gulam Abad Hospital (GMH) Maqsood-ul-Zia Hospital (MZH), Mian Trust Hospital (MtH).

Table 01: Frequency of Gram-negative bacteria isolated from Hospital Wastewater and Community Wastewater

Gram-negative Bacteria (n=165)		Hospital Waste Water (n=79)	Community Waste Water (n=86)
E.coli	(n=64)	48.1%	30.3%
K. pneumonia	(n=48)	25.3%	32.6%
Shigella species.	(n=25)	8.9%	20.9%
Pseudomonas aeruginosa (n=20)		12.7%	11.6%
Serratiamarcescens	(n=5)	3.8%	2.3%
Salmonella species.	(n=3)	1.3%	2.3%

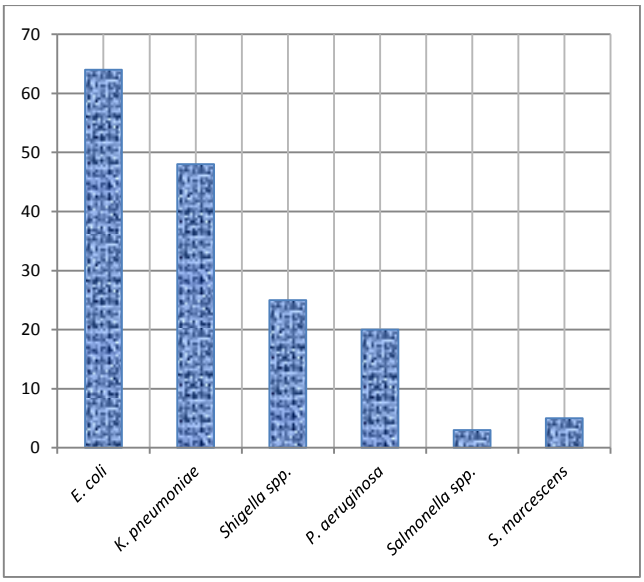

Figure 03: Total isolated Gram-negative bacteria in a study (n=165)

Table 02: Percentage of Antibiotic-resistant Gram-negative bacteria isolated from Hospital Wastewater against commonly available antibiotics

Antibiotics	E.coli (n=38)	K. pneumonia (n=20)	Shigellaspecies (n=07)	P. aeruginosa (n=10)
SAM	71.05%	22.01%	42.85%	40.00%
CRO	100%	10.48%	14.28%	10.00%
FEP	100.0%	10.00%	14.28%	10.00%
STX	34.21%	55.34%	14.28%	20.00%
TZP	50.00%	35.24%	28.57%	20.00%
CIP	73.68%	20.34%	42.85%	50.00%
AK	26.31%	5.09%	14.28%	20.00%
MEM	47.36%	95.00%	14.28%	10.00%
TGC	94.73%	100.0%	71.42%	100.0%
PB	2.14%	5.14%	14.28%	10.00%
CT	10.52%	5.03%	14.28%	10.00%

Abbreviations; Ampicillin sulbactam (SAM), Ceftriaxone (CRO), Cefepime (FEP), Trimethoprim-sulfamethoxazole (STX), Piperacillin-tazobactam (TZP), Ciprofloxacin (CIP), Amikacin (AK), Meropenem (MEM), Tigecycline (TGC), Polymyxin B (PB) and Colistinsulfate (CT).

Table 03: Percentage of Antibiotic resistance in Gram-negative bacteria inCommunity Wastewater against commercially available antibiotic disks

Antibiotics	E.coli n=(26)	K. pneumonia n=(28)	Shigella species n=(18)	P. aeruginosa n=(10)
SAM	65.38%	25.00%	77.78%	80.00%
CRO	80.76%	21.42%	5.55%	10.00%
FEP	100.0%	3.57%	5.55%	10.00
STX	38.46%	14.28%	11.12%	10.00%
TZP	57.69%	28.57%	44.45%	60.00%
CIP	76.92%	28.57%	38.88%	10.00%
AK	3.84%	7.14%	5.55%	30.00%
MEM	42.30%	25.00%	33.34%	10.00%
TGC	94.73%	100.0%	100.0%	80.0%
PB	3.84%	3.57%	5.55%	10.00%
CT	7.69%	7.14%	5.55%	10.00%

Abbreviations; Ampicillin sulbactam (SAM), Ceftriaxone (CRO), Cefepime (FEP), Trimethoprim-sulfamethoxazole (STX), Piperacillin-tazobactam (TZP), Ciprofloxacin (CIP), Amikacin (AK), Meropenem (MEM), Tigecycline (TGC), Polymyxin B (PB) andColistinsulfate (CT).

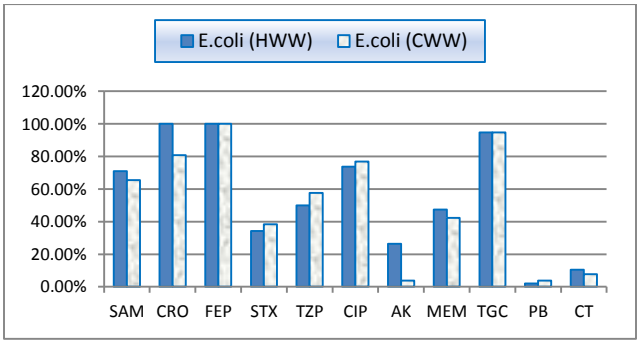

Figure 04: Compare resistance pattern of E.coli isolated from hospital wastewater (HWW) and community wastewater (CWW)

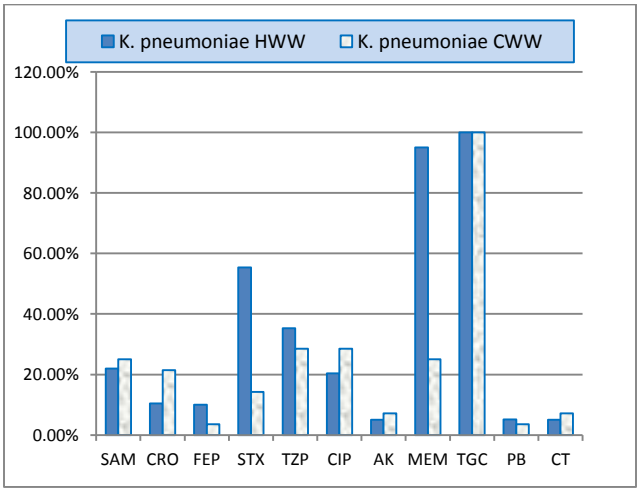

Figure 05: Compare resistance pattern of K. pneumonia isolated from hospital wastewater (HWW) and community wastewater (CWW)

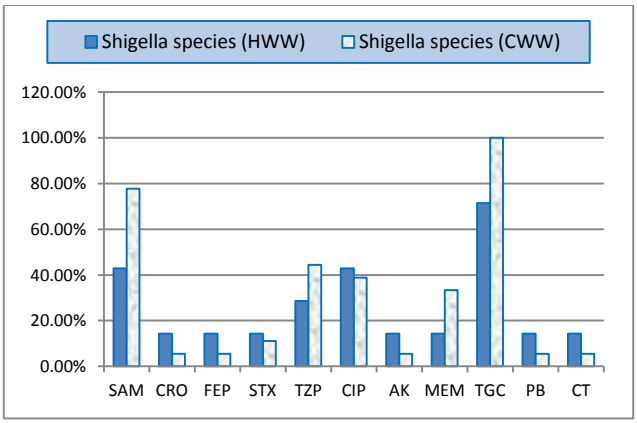

Figure 06: Resistance pattern of Shigellaspeciesisolated from hospital wastewater (HWW) and community wastewater (CWW)

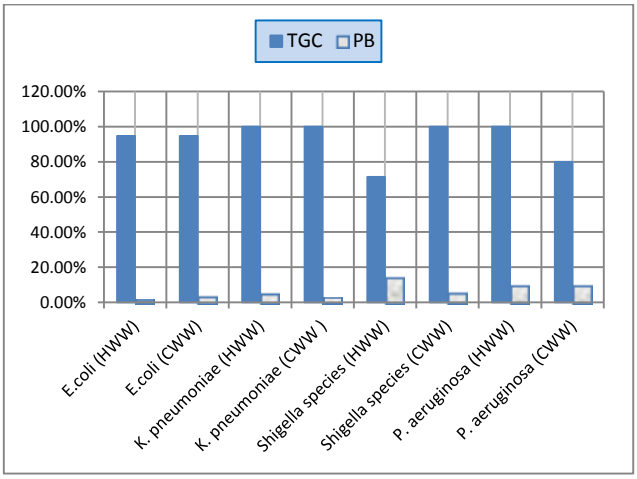

Figure 07: Comparison of Tigecycline (TGC) and Polymyxin B (PB) resistance in marked isolated bacteria from hospital wastewater (HWW) and community wastewater (CWW) Abbreviations; Ampicillin sulbactam (SAM), Ceftriaxone (CRO), Cefepime (FEP), Trimethoprim-sulfamethoxazole (STX), Piperacillin-tazobactam (TZP), Ciprofloxacin (CIP), Amikacin (AK), Meropenem (MEM), Tigecycline (TGC), Polymyxin B (PB), and Colistinsulfate (CT).

Genetic Analysis of Enterobacteriaceae based on blaCTX-M: All isolated bacterial DNA was isolated for the identification of blaCTX-M. The presence of these genes was confirmed by using PCR. Amplifies bands were visualized through agarose gel electrophoresis followed by a gel documentation system (UV illuminator). A 100bp leader was used for marking genes based on their specific sequence size. The bands of blaCTX-M were confirmed as 593bp. A total of 50 bacteria were positive for blaCTX-M, among whichE.coli (n=26) was the most important bacteria noted having blaCTX-M followed by K. pneumonia (n=12), Shigella species (n=5), Pseudomonas aeruginosa(n=5)and Serratiamarcescens (n=2) (Figure 08).

CTX-M-positive bacteria were found to be most resistant to most of the applied antibiotics, especially against CTX-M-positive E.coli and K. pneumonia. The data of antimicrobial susceptibility patterns against E.coli, K. pneumonia, P.aeruginosa, and Shigella species are summarized in Table 4.5. Ampicillin sulbactam, ceftriaxone, cefepime, ciprofloxacin, meropenem, and tigecycline were observed to be potentially resistant antibiotics against CTX-M producer organisms as compared to non-CTX-M Gram-negative bacteria. The CTX-M producers E.coli were 100% resistant to cefepime while CTX-M producers K. pneumonia, P.aeruginosa, and Shigella species were 100% resistant against tigecycline. The total CTX-M producers of E.coli were resistant to ampicillin-sulbactam (72.0%), ceftriaxone (80.0%), cefepime (100%), ciprofloxacin (76.92), meropenem (40.0%) and tigecycline (96%). The CTX-M producers K. pneumonia were leading resistant against meropenem (55.55%), and tigecycline (100.0%). The CTX-M producer's Shigella species were predominantly resistant to ampicillin-sulbactam (100.0%), ciprofloxacin (60.0%), meropenem (40.0%), and tigecycline (100.0%). The CTX-M producers P.aeruginosa were noted to be prominent resistant to ampicillin-sulbactam (80.0%), piperacillin-tazobactam (40.0%), and tigecycline (100.0%).

Interestingly, tigecycline was observed as a predominant resistant (> 96%) antibiotic followed by meropenem (> 40%) against all isolated CTX-M producers. The resistance to trimethoprim-sulfamethoxazole, piperacillin-tazobactam, amikacin, and colistin sulfate was conversely less frequently resistant to CTX-M producers (Table 4.5). Overall, the data of antibiotic-resistant to CTX-M producers E.coli again predominant observed while less frequent resistant in K. pneumonia against all applied antibiotics. The identification of CTX-M in Enterobacteriaceae has been noted as having a pivotal role in the production of β-lactamases. This CTX-M has been linked with various types of mobile genetic elements that cause rapid and proficient inters replicon along with cell-to-cell distribution of antibiotic-resistant (D'Andrea et al., 2013). This elevated multiresistant species of Enterobacteriaceaehad spread on a large scale in the presence of hospital wastewater.

Table 04: Showing the frequency of bla CTX-M Positive gene

Isolated Gram-negative bacteria (n=165)		CTX-M Positive (n=50)	Percentage (30.31%)
From Hospital Wastewater	(n=79)	23	29.12%
From Community Wastewater	(n=86)	27	31.39%

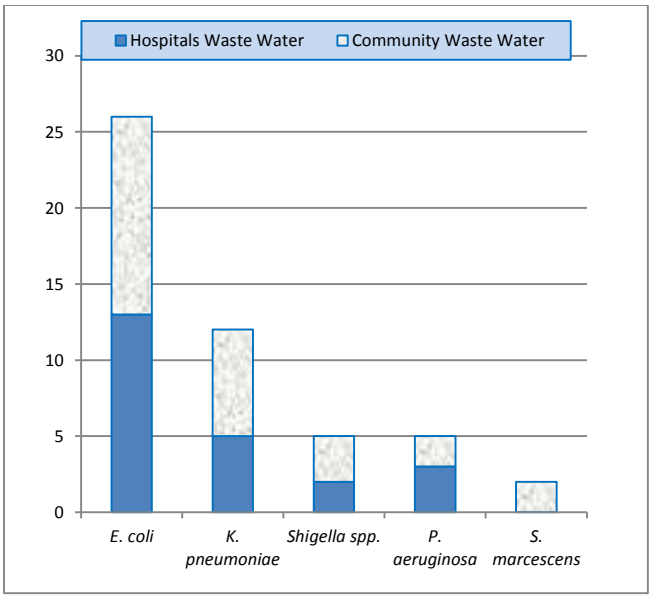

Figure 08: Frequency of Gram-negative bacteria for blaCTX-M in a study (n=50)

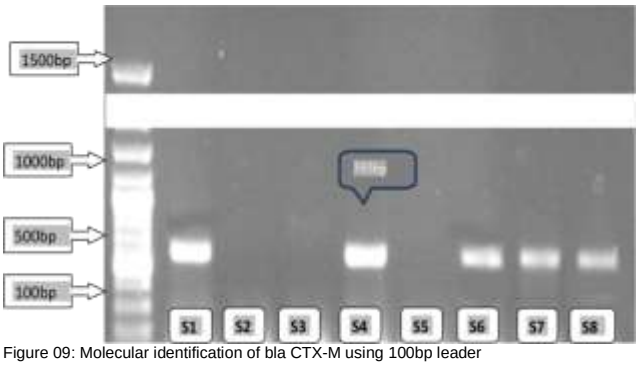

Figure 09: Molecular identification of bla CTX-M using 100bp leader

Table 05: Comparison the AST 0f CTX-M and Non CTX-M Gram-negative bacteria

Antibiotics	E.coli		K. pneumonia		Shigellaspecies		P. aeruginosa	
	Non CTX-M	CTX-M	Non CTX-M	CTX-M	Non CTX-M	CTX-M	Non CTX-M	CTX-M
SAM	65.78%	72.00%	36.11%	33.33%	68.42%	100.0%	53.33%	80.00%
CRO	100.0%	80.00%	11.11%	25.00%	5.26%	2.70%	13.33%	2.04%
FEP	100.0%	100.0%	2.77%	8.33%	2.07%	3.20%	3.76%	5.89%
STX	31.57%	40.00%	27.77%	41.66%	10.52%	2.47%	5.55%	20.00%
TZP	60.52%	48.00%	33.33%	33.33%	42.10%	40.00%	40.00%	42.89%
CIP	71.05%	76.92%	19.44%	33.33%	31.57%	60.00%	35.34%	20.0%
AK	23.68%	28.00%	8.33%	2.08%	10.52%	2.28%	26.66%	20.00%
MEM	47.36%	40.00%	55.55%	50.00%	26.31%	40.00%	6.67%	3.78%
TGC	94.73%	96.00%	91.66%	100.0%	94.73%	100.0%	86.66%	100.0%
PB	2.63%	1.99%	2.77%	1.07%	2.77%	3.02%	2.89%	3.94%
CT	7.84%	8.00%	2.78%	8.33%	2.77%	3.48%	2.44%	3.07%

DISCUSSION

Throughout this study, E.coli was noted as the most important Gram-negative bacteria having multidrug-resistant against almost all applied antibiotics to proliferate antibiotic resistance among Enterobacteriaceae species from hospital wastewater as well as community wastewater.The second most important bacteria were K. pneumonia followed by Shigella species, P.aeruginosa, Salmonella species, and S. marcescens isolated from hospital and community wastewater. The publication of Rio de Janeiro, Brazil also revealed that E.coli and K. pneumonia had been identified as the most common ESBL producers (Chagas, Seki, Cury, et al., 2011). Another study presented multidrug-resistant P.aeruginosa (MDR-P) as related to environmental contamination even with tap water (Breathnach, Cubbon, Karunaharan, Pope, & Planche, 2012). All isolates were identified by Chrome agar and their respective biochemical tests were finally then sensitized for determining the resistant pattern against their respective antibiotics.An American journal concluded that β-lactams antibiotics were extensively prescribed for hospital-admitted patients to treat infectious diseases against species of Enterobacteriaceae. Later at the end, these metabolites and other active compounds were isolated again from the hospital wastewater which was considered a medium to share these β lactamases and extended spectrumβ-lactamases genes(Korzeniewska& Harnisz, 2013).Rafeel Canton explained the association of CTX-M to ESBLthatisentrenched by class 1 integrons. Thefindings of bla CTX-M were noticed in most of the species of Enterobacteriaceae and other Gram-negative bacteria which led to MDR(Cantón& Coque, 2006).

The AST was done by using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar through CLSI guidelines 2016. All isolated E.colifrom hospital and community wastewater were 100% resistant against cefepime and 94.73% against tigecycline. InE.coli, the variable resistance pattern of ciprofloxacin was 73.68% against isolates from hospital wastewater and 76.92% against isolates from community wastewater. A study on community wastewater found the E.coli resistant against tigecycline (52%)(Watkinson, Micalizzi, Graham, Bates, & Costanzo, 2007). Another study fromtheeffluent of hospitals reported ciprofloxacin (12.8%) and cefepime (18.8%) against isolated E.coli(Picão et al., 2013).All isolated K. pneumonia from hospital and community wastewater was 100% resistanttotigecycline. In K. pneumonia, the variable resistance pattern of meropenem was 95.00% resistance and trimethoprim-sulfamethoxazole was 55.34% resistance against isolates from hospital wastewater. An observation on the occurrence of K. pneumonia in hospital wastewater showed resistanceto trimethoprim-sulfamethoxazole associated with CTX-M (Chagas, Seki, Da Silva, & Asensi, 2011). For Shigellaspecies, the variable resistant pattern of tigecycline was 71.42% while42.85% resistant against ciprofloxacin and ampicillin-sulbactam from hospital wastewater. All isolated Shigellaspecies from community wastewater were 100% resistant against tigecycline while 77.78% against ampicillin sulbactam. A study on drug resistant showed ampicillin sulbactam (50%) against Shigella species isolated from hospital wastewater (Moges, Endris, Belyhun, & Worku, 2014). All isolated P.aeruginosa from hospital wastewater were 100% resistant against tigecycline while 50.0% resistant against ciprofloxacin. InP.aeruginosa, the variable resistance pattern of ampicillin-sulbactam and tigecycline was 80.00% while piperacillin-tazobactam was 60.0% against isolates from community wastewater. A study has revealed the role P.aeruginosa to drugs resistant through hospital wastewater and showed pattern of drugs resistant 12% against ciprofloxacin and piperacillin(Miranda et al., 2015). Although, the concentration of antibiotics found in hospital wastewater was usually low than the applied concentration of antibiotics on the sensitivity plate. There was probably difficult for us to determine the exact resistant pattern of suspected bacteria. The data of resistant pattern of isolates from hospital wastewater of Faisalabad showed diverse and extensive results as compare to other studies in developed countries.

About 30.3% of bla CTX-M genes were identified in this study.E.coli and other species of Enterobacteriaceae species share CTX-M by horizontal gene transfer mechanism through resistant genes in presence of hospital wastewater as a medium. All isolated EnterobacteriaceaeandP.aeruginosawere amplified for the identification of blaCTX-M using forward and reverse primers. Out of 165 isolated bacteria, 50 Gram-negative bacteria were identified as CTX-M producers. Here E.coli (n=26)was again the most important bacteria noted having blaCTX-M followed by K. pneumonia (n=12), Shigella species (n=5), Pseudomonas aeruginosa(n=5)and Serratiamarcescens(n=2).The Enterobacteriaceae species having extended-spectrum β-lactamases (ESBLs) were genetic characteristics of most Enterobacteriaceae, especially among E.coli.The epidemiology of ESBL-positive E.coli in this study showed predominant strains and enhanced diversity of clones over time. A result of the study found an association of CTX-M with ESBL in K. pneumonia, P.aeruginosa, and S. marcescensandShigella species (Pitout & Laupland, 2008).The trend of remaining ESBL-positive strains like K. pneumonia, Shigella species, Pseudomonas aeruginosa, and Serratiamarcescens were also notable in our study. The mutation in genes of clonal bacteria enhances the burden of broad-spectrum antibiotic resistance like CTX-M mostly in E.coli(Jacoby & Carreras, 1990).So, these results of data indicated both epidemiological transfers of genetic components and clonal spread which might be considered partners for exploring of ESBLs.The trends of blaCTX-M-positive bacteria isolated from hospital and community wastewater were almost the same. Genotypical investigation revealed the underlying resistance mechanism to differentiate intrinsically and acquired antibiotic resistance in hospital wastewater. The detection of bla CTX-M resistance genes in the Enterobacteriaceae family from hospital wastewater concerned the impact on the genotype of the Enterobacteriaceaefamily from community wastewater. Other resistance genes could be detected in these isolates.

The hospital wastewater played an emergency role in antibiotic resistance and then contributed to environmental bacteria in community wastewater. Hospital wastewater provided an advantage for the persistence and multiplication of Enterobacteriaceae such as increased temperature, pH, metabolites, and non-metabolites of different antibiotics (Baquero, Martínez, & Cantón, 2008). High densities of bacteria in hospital

wastewater got support for sharing resistance genes horizontally among them through plasmid-carrying resistance genes like R plasmid (Williams et al., 2000). Most of the Gram-negative bacteria especially the family of Enterobacteriaceaewas able to share genes in this environment.

The results of the whole study were expected to be helpful to realize that hospital wastewater would be treated through wastewater treatment plants (WWTPs) before exposing waste to community wastewater. Although community wastewater was already polluted with the highest resistant Enterobacteriaceae and other Gram-negative bacteria species, it could be minimized by using wastewater treatment plants in hospitals in the future. Worldwide, hospital effluents were burdened with ARB but only a few countries recommend specific treatment plants to treat hospital wastewater before exposing it to community wastewater.

CONCLUSION

The study indicated the percentage of antibiotic resistance to bacteria from hospital wastewater wasmore variable than those found from community wastewater. Interestingly, most of these strains had an identical pattern of antibiotic resistance from hospital and community wastewater.All isolated are predominantly noted as MDR bacteria might be due to producers of CTX-M, which are found to be associated with multidrug-resistant such as resistant to ampicillin-sulbactam, ceftriaxone, cefepime, ciprofloxacin, meropenem, and tigecycline. Tigecycline was observed as a predominant resistant (>96%) antibiotic followed by meropenem (>40%) against all isolated CTX-M producers. The resistance to trimethoprim-sulfamethoxazole, piperacillin-tazobactam, amikacin, and colistinsulfate was conversely less frequently resistant among CTX-M producers. CTX-M producers were identified among both isolates from hospital and community wastewater. The antimicrobial susceptibility testing of CTX-M was also predominantly resistant as compared to Non-CTX-M bacteria. The conclusion and observation represented the existence of MDR Enterobacteriaceae in untreated hospital wastewater. These bacteria had a chance to travel to the inlet of community wastewater without any wastewater treatment. So, it is a significant risk for public health to acquire these strains of bacteria through contaminated foods and drinks.

Declaration of conflicting interests: The author(s) declared no potential conflicts of interest with respect to the research, authorship,and/or publication of this article.

Funding: This study was not funded by any organization.

Disclosure statement: The authors declare no conflict of interest.

Ethical compliance: This article does not contain any studies involving human participants or animals performed by any of the authors.

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