Prevalence of AmpC and Extended-Spectrum Beta-Lactamase Producing E. coli and Klebsiella spp. in Sewage Effluents of Dharan, Nepal

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Research Article

Keywords: ESBL, ABL, Enterobacteriaceae, E. coli, Klebsiella spp, Sewage effluent

Posted Date: April 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1509782/v1

Abstract

Background

The prevalence of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase (ABL) producing *Enterobacteriaceae* is increasing rapidly across the world. Members of *Enterobacteriaceae* like *E. coli* and *Klebsiella spp*. exhibit antimicrobial resistance mainly due to the production of beta-lactamase enzymes like extended-spectrum βlactamases, AmpC β-lactamases, and carbapenemases. These bacteria are frequently reported in sewage effluents of hospital and municipal sewerage systems indicating sewage as a promising source for dissemination of such drug-resistant pathogens. However, in most of the developing countries including Nepal, the major portion of sewage is discharged in water sources without proper treatment and disinfection. This study was undertaken to access the prevalence of ESBL and ABL producing *E. coli* and *Klebsiella spp*. in sewage effluents of Dharan, Nepal.

Results

A total of 235 bacteria were isolated, out of which 103 (43.83%) were *E. coli* and 132 (56.17%) were *Klebsiella spp*. ESBL production was seen in 157 (66.81%) isolates. Among them, 89 (56.69%) were *Klebsiella spp*. and 68 (43.31%) were *E. coli.* 66.02% of total isolated *E. coli* and 67.42% of total isolated *Klebsiella spp*. showed production of ESBL enzymes. ABL production was seen in 133 (56.59%) isolates. Among them, 54 (40.60%) were *E. coli* and 79 (59.40%) were *Klebsiella spp.* 52.43% of the isolated *E. coli* and 59.85% of isolated *Klebsiella spp*. were found producing ABL enzyme.

Conclusions

The results indicate that there is a high prevalence of ESBL and ABL producing *E. coli* and *Klebsiella spp*. in sewage effluents of Dharan. Effective treatment of sewage effluents must be ensured before discharging the sewage into the environment. National guidelines for discharging the municipal sewage must be immediately amended and an effective treatment system before discharge must be implemented. Dissemination of such drug-resistant bacteria in the human population leading to severe public health emergency is likely to occur from sewage contamination, so further study and surveillance and effective prevention and control measures are necessary.

Background

Sewage is the collection of wastewater in a municipal piper system or sewerage channel. It is mostly a combination of domestic effluents from toilets, bathing, and kitchen, water from commercial establishments and institutions including hospitals and clinics, hotels and restaurants, shops and markets, schools, colleges, and different offices, industrial effluents, and surface runoff water (Mateo-Sagasta et al., 2015). Sewage water contains a different variety of organic and inorganic wastes and

nutrients merged from different sources. Hospital sewage water is loaded with several pathogenic microorganisms with antimicrobial resistance capacity, partially metabolized pharmaceutical substances like antimicrobial, pharmaceutical, disinfectants, and un-metabolized drugs, radioactive elements, and other toxic substances. Non-hospital sewage is loaded with microorganisms from human and animal feces and environmental sources. This will make sewage more prone to pathogenic organisms including antibiotic-resistant bacteria (Mahato et al., 2019). Different studies have shown the presence of antibiotic-resistant bacteria and genes which have even escaped treatment systems and disseminated in wastewater (Nasser et al., 2019). Wastewater treatment systems can reduce contaminants including microorganisms to a minimum limit, but only 5% of generated wastewater is treated appropriately while the remaining is directly connected to water bodies in Nepal. This has resulted in the transmission of microorganisms and water-borne infections (Jha & Bajracharya, 2014).

Enterobacteriaceae covers a large part of hospital sewage microbiota (Korzeniewska & Harnisz, 2013). Among the sewage pathogens, coliforms are responsible for frequent human infections (Doi et al., 2017). Coliforms are aerobic or facultative, Gram-negative, non-sporing lactose fermenting bacteria. They were traditionally represented by 4 genera viz. *Escherichia, Klebsiella, Citrobacter*, and *Enterobacter*, but now contain over 20 bacterial genera (Masiello et al., 2016). *Enterobacteriaceae* includes different Gram-negative bacteria including pathogenic genera like *Escherichia, Salmonella, Shigella, Klebsiella*, and *Serratia* (Rock & Donnenberg, 2014). *Klebsiella pneumoniae* and *E. coli* of *Enterobacteriaceae* have notable antibiotic resistance to a wide variety of antibiotics typically used in the treatment of their infection (Le et al., 2016; Kazemian et al., 2019).

Members of *Enterobacteriaceae* like *E. coli* and *Klebsiella* spp. exhibit antimicrobial resistance mainly due to the production of beta-lactamase enzymes like extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases (Ojer-Usoz et al., 2013). *Enterobacteriaceae* were found to produce intrinsic chromosomal encoded beta-lactamases but due to the transfer of such gene to transferable plasmid gene, there is rapid dissemination of *Enterobacteriaceae* resistant to a wide variety of beta-lactam antibiotics (Susić, 2004).ESBLs are *β*-lactamases capable of developing resistance against *β*-lactam drugs like penicillins, extended-spectrum cephalosporins, and monobactams except for cephamycins and carbapenems and susceptible to *β*-lactam inhibitor clavulanic acid (Raut et al., 2015). ABL is characterized by the hydrolysis of broad-spectrum cephalosporins like cefotaxime and ceftazidime and cephamycins (7-α-methoxy cephalosporins) like cefoxitin and cefotetan, including monobactam, and the β-lactam/β-lactam inhibitor combination, but are sensitive to cefepime and carbapenems (Dehkharghani et al., 2020).

Different clinical isolates of *E. coli* and *Klebsiella spp*. are found to be antibiotic resistant and produce ESBL and ABL enzymes for conferring the resistance against *β*-lactam antibiotics. (Shakya et al., 2017) concluded on the higher prevalence of ESBL producing *E. coli* and *Klebsiella spp*. in Nepal by examining 2209 non-repetitive MSU samples at a tertiary hospital of Nepal. Similarly, (Baral et al., 2013) and (Aryal et al., 2020)concluded the high prevalence of ABL producers in clinical isolates. (Mahato et al., 2019)

studied sewage effluents of different hospitals of Biratnagar, Nepal, and concluded the prevalence of MDR and ESBL producing *E. coli* and *Klebsiella spp*. in hospital sewage effluents.

Sewage effluents have high potentiality as an environmental reservoir of a wide variety of pathogenic organisms including ESBL and ABL producing *E. coli* and *Klebsiella spp.* Unfortunately, scanty research is done on sewage effluents as a source of such pathogens. The present study was undertaken to reduce the research gap on assessment of sewage microbiota with the sole focus on determining the prevalence of ESBL and ABL producing *E. coli and Klebsiella spp.* in sewage effluents of Dharan, Nepal.

Results

Distribution of E. coli and Klebsiella spp. in the Samples

Among the total of 20 samples analyzed 16 samples (80%) showed growth of either *E. coli* or *Klebsiella spp*. or both and 4 samples (20%) did not show growth of *E. coli* and *Klebsiella spp*.(Table 1). Out of 16 positive samples, 13 (81.25%) showed growth of both *E. coli* and *Klebsiella spp*., 2 (12.5%) showed growth of *Klebsiella spp*. only and 1 (6.25%) showed growth of *E. coli* only. All of the hospital-sewage effluents (6 out of 6) and 8 of the non-hospital sewage effluents (8 out of 14) showed growth of both *E. coli* and *Klebsiella spp*.(Table 1). This showed that 70% of the samples contained *E. coli* and 75% of the samples contained *Klebsiella spp*.

Table 1
Prevalence of *E. coli* and *Klebsiella spp*. in each sample

Sample	Location	Bacteria Isolated	
		E. coli	Klebsiella spp.
1	BPKIHS, Dharan	+	+
2	BPKIHS, Dharan	+	+
3	BPKIHS, Dharan	+	+
4	BPKIHS, Dharan	+	+
5	Bijaypur Hospital Pvt.Ltd	+	+
6	Bijaypur Hospital Pvt. Ltd	+	+
7	Residential area – 1	-	-
8	Residential area – 2	+	+
9	Residential area – 3	-	+
10	Residential area – 4	-	-
11	Market area – 1	+	+
12	Market area – 2	+	+
13	Market area – 3	+	+
14	Commercial area – 1	+	+
15	Commercial area – 2	+	+
16	Commercial area – 3	+	-
17	Commercial area – 4	-	-
18	Commercial area – 5	-	+
19	Commercial area – 6	-	-
20	Commercial area – 7	+	+

A total of 235 bacteria were identified as either *E. coli* or *Klebsiella spp*. Out of them, 103 isolates (43.83%) were *E. coli* and 132 isolates (56.17%) were *Klebsiella spp*. The prevalence of *Klebsiella* was found to be higher. (Fig. 1)

Antibiotic Susceptibility Pattern of Isolated E. coli **and** Klebsiella spp.

Antibiotic sensitivity test of selected antibiotics against the isolated *E. coli* and *Klebsiella spp*. showed the highest sensitivity to Azithromycin (63.82%) and the lowest sensitivity to Cefoxitin (1.28%) (Table 2).

Table 2

Antibiotic Susceptibility Pattern of Isolated E. coli **and** Klebsiella spp.

Antibiotics	Disc Content (in \varvec$\mu$\varvec$g$)	Susceptibility Pattern	
		Sensitive n (%)	Resistant n (%)
Ampicillin	10	27 (11.49%)	208 (88.51%)
Azithromycin	15	150 (63.83%)	85 (36.17%)
Aztreonam	30	55 (23.40%)	180 (76.60%)
Ciprofloxacin	5	113 (48.09%)	122 (51.91%
Co-Trimoxazole	25	146 (62.13%)	89 (37.87%)
Ceftriaxone	30	45 (19.15%)	190 (80.85%)
Ceftazidime	30	39 (16.59%)	196 (83.41%)
Cefotaxime	30	41 (17.45%)	194(82.55%)
Cefoxitin	30	3 (1.28%)	232 (98.72%)
Imipenem	10	140 (59.57%)	95 (40.43%)
Nitrofurantoin	300	127 (54.04%)	108 (45.96%)

Beta-lactam antibiotics; Ampicillin, Aztreonam, Ceftriaxone, Ceftazidime, Cefotaxime, and Cefoxitin showed very poor sensitivity patterns showing the sensitive result to only 11.49%, 23.40%, 19.15%, 16.59%, 17.45%, and 1.28% of tested bacteria respectively. Ciprofloxacin showed sensitivity to 48.09% of the tested bacterial species, while Co-Trimoxazole showed sensitivity to 62.13% of the tested bacterial species. Imipenem showed a sensitive result on 59.57% of the tested bacteria. Nitrofurantoin showed a sensitive result on 54.04% of the tested bacteria.

Antibiotic susceptibility test of *E. coli* revealed that 84.47% of them were sensitive to Nitrofurantoin, while only 2.91% were sensitive to Cefoxitin. Ampicillin, Azithromycin, Aztreonam, Ciprofloxacin, Co-Trimoxazole, Ceftriaxone, Ceftazidime, Cefotaxime, and Imipenem showed sensitivity against 15.53%, 55.34%, 31.07%, 48.54%, 68.93%, 31.07%, 25.24%, 20.39%, and 58.25% of the tested *E. coli* respectively.

On other hand, 70.45% of *Klebsiella spp*. were sensitive to Azithromycin and 0% of *Klebsiella spp*. were sensitive to Cefoxitin. Ampicillin, Aztreonam, Ciprofloxacin, Co-Trimoxazole, Ceftriaxone, Ceftazidime, Cefotaxime, Imipenem, and Nitrofurantoin showed sensitivity against 8.33%, 17.42%, 47.73%, 56.82%, 9.85%, 9.85%, 15.15%, 60.61%, and 30.31% of the tested *Klebsiella spp*. respectively.

Distribution of ABL Producing E. coli **and** Klebsiella spp.

133 isolates (56.59%) were found positive for ABL production. Among 133, 54 (40.60%) were *E. coli* and 79 (59.40%) were *Klebsiella spp*. (Fig. 2) This showed a higher prevalence of ABL producing *Klebsiella spp.* than *E. coli* in sewage effluents of Dharan.

Distribution of ESBL Producing E. coli **and** Klebsiella spp.

ESBL production was seen in 157 isolates (66.81%) while testing 235 isolated bacteria. Out of 157 ESBL producing 89 (56.69%) were *Klebsiella spp*. and 68 (43.31%) were *E. coli*. (Fig. 3) This showed a higher prevalence of ESBL producing *Klebsiella spp*. than *E. coli* in sewage effluent of Dharan.

Distribution of ABL and ESBL Co-producing E. coli **and** Klebsiella spp.

A total of 118 isolates showed the production of both ABL and ESBL enzymes. This accounted for 50.21% of total isolated bacteria. Out of 118 co-producers, 51 (43.22%) were *E. coli*, and the remaining 67 (56.78%) were *Klebsiella spp*. (Fig. 4) Prevalence of ABL and ESBL co-production was comparatively higher in *Klebsiella spp*. than in *E. coli.*

Discussion

Beta-lactam antibiotics are among the most widely used antibiotic class(Bush & Bradford, 2016). Bacteria have developed resistance to β-lactam antibiotics by evolving their ability to synthesize several types of β -lactamase enzymes capable of hydrolyzing β-lactam ring. Among the β-lactamase enzymes ESBL and ABL are the most commonly encountered types (Bush & Bradford, 2020, Bush & Jacoby, 2010). Resistance to β-lactam antibiotics by producing ESBL and ABL enzymes is increasing rapidly among members of *Enterobacteriaceae* across the world(Caron et al., 2018) (De Angelis et al., 2020). This study was conducted to detect the prevalence of ABL and ESBL producing *E. coli* and *Klebsiella spp.* in sewage effluents of Dharan. A total of 20 samples were analyzed in the Molecular and Microbiology Laboratory of Sunsari Technical College, Dharan-1 from May 21, 2021, to October 29, 2021. The phenotypic method was used to confirm the production of AmpC Beta-lactamase and Extended Spectrum Beta-lactamase enzymes in the isolated species using the methods recommended by CLSI(Patel & Clinical and Laboratory Standards Institute, 2017)

Among the total of 20 samples analyzed 16 samples (80%) showed growth of either *E. coli* or *Klebsiella spp*. or both and 4 samples (20%) did not show growth of *E. coli* and *Klebsiella spp*. Among the studied sample, 70% (14 out of 20) showed the presence of *E. coli*, and 75% (15 out of 20) showed the presence of *Klebsiella spp*. Mahato et al., 2019 had reported that 70% of sewage effluents contained *E. coli* while only 60% of the sewage effluents contained *Klebsiella spp*. This variation may be since Mahato et al., 2019 had studied hospital sewage effluents only while we studied both hospital and municipal sewage.

In the study, 235 bacteria were identified as either *E. coli* or *Klebsiella spp.* 103 (43.83%) of them were *E. coli*, whereas 132 (56.17%) of them were *Klebsiella spp*. Results obtained by Mahato et al., 2019 were different. They had shown that 53.85% of their isolates were *E. coli*, while 46.15% were *Klebsiella spp*.

(Fadare & Okoh, 2021) had shown that 65.71% of their isolates were *Klebsiella spp*. and only 10% were *E. coli*.

Ampicillin resistance was seen in 84.47% of *E. coli*. It was 44.66% for Azithromycin, 68.93% for Aztreonam, 51.46% for Ciprofloxacin, 31.07% for Co-Trimoxazole, 68.93% for Ceftriaxone, 74.76% for Ceftazidime, 79.61% for Cefotaxime, 97.09% for Cefoxitin, 41.75% for Imipenem, and 15.53% for Nitrofurantoin. This result was similar to Mahato et al., 2019 for Ampicillin, Aztreonam, and Cefoxitin. They had shown 100% resistance to these antibiotics. But, for Co-Trimoxazole, Ceftazidime, Ceftriaxone, and Cefotaxime they had shown resistance to 85.7% which was higher than the result obtained in this research. Similarly, they had shown that resistance to Nitrofurantoin was 42.9%, which was higher in comparison to this research. For Azithromycin, they had shown that non-of them (0%) were resistant, but 44.66% were found resistant in this research. They had reported that 14.3% were found to be resistant to Ciprofloxacin but it was 51.46% in this research.

This study showed that 91.67% of *Klebsiella spp*. were resistant to Ampicillin. It was 29.55% for Azithromycin, 82.58% for Aztreonam, 52.27% for Ciprofloxacin, 43.18% for Co-Trimoxazole, 90.15% for Ceftriaxone, 90.15% for Ceftazidime, 84.85% for Cefotaxime, 100% for Cefoxitin, 39.39% for Imipenem, and 69.69% for Nitrofurantoin. The result was shown by Mahato et al., 2019 indicates comparatively higher resistance against Ampicillin and Co-Trimoxazole, and lower to the other antibiotics.

ESBL production was found higher in *Klebsiella spp*. than in *E. coli*. Out of 157 ESBL producers, 43.31% (68) were *E. coli*. and 56.69% (89) were *Klebsiella spp*. Mahato et al., 2019 showed higher ESBL production in *E. coli* (66.67%) than in *Klebsiella spp*. (33.33%). Similarly, Zaatout et al., 2021 also had reported a higher prevalence of ESBL *E. coli* in sewage effluents than that of *Klebsiella spp*. Fadare & Okoh, 2021 on the other hand had shown a higher prevalence of ESBL producing *Klebsiella spp*. than *E. coli* in hospital wastewater. ESBL production was seen in 81.59% of total tested *E. coli* and 85.61% of total tested *Klebsiella spp*. A higher prevalence of ESBL producing *E. coli* was found by (Reinthaler et al., 2009).

ABL production was also found to be higher in *Klebsiella spp*. than in *E. coli*. Among 133 ABL producers, 59.40% (79) were *Klebsiella spp*. and 40.60% (54) were *E. coli*. Among 57 wastewater samples tested by (Ben Said et al., 2016), 24 were found to be positive for ESBL-Eb or pAmpC-Eb producing *Enterobacteriaceae*. They detected ESBL-Eb in 20 samples, and pAmpC-Eb in 4 samples. No relevant literature on ABL producing *E. coli* and *Klebsiella spp*. in sewage effluents were found for further comparison.

ESBL and ABL co-production was seen in 50.21% (total 118) of the isolates. 75% of the ESBL producing *E. coli* were found to produce ABL also, and 75.28% of the ESBL producing *Klebsiella spp*. were found to produce ABL also. This result was higher than that of Rizi et al., 2020. They had shown co-production in only 30% of the isolated *E. coli* and *Klebsiella spp*. Similarly, co-production was seen in 29% of the isolated *E. coli* and *Klebsiella pneumoniae* in research by (Hertz et al., 2019). Co-production was seen in

9.38% of isolated *E. coli* and non (0%) of isolated *Klebsiella spp*. by (Shrestha et al., 2019). These variations may be because all these studies are on clinical isolates.

Conclusions

Based on the finding of the present study, it can be concluded that sewage effluents of Dharan have a higher prevalence of ESBL and ABL producing *E. coli* and *Klebsiella spp*. This suggests that there may be a direct link between human or animal feces in the sewage system. The result is an alarming indicator of a probable outbreak of life-threatening infection by *β*-lactamase producing *E. coli* and *Klebsiella spp*. if sewage is contaminated with drinking water channels or food in the Dharan area. There is an urgent need for further research in sewage microflora and the probability of their dissemination to human populations. Government bodies should prepare effective regulatory measures to control the irrational use of antibiotics and monitor the antimicrobial resistance status of different suspected bacteria. Effective alternatives for the treatment of ESBL and ABL producing *E. coli* and *Klebsiella spp*. may be needed soon. Immediate and effective response from concerned authorities for controlling the dissemination of such bacteria from sewage to the environment or community is required. Regular monitoring and study of antibiotic susceptibility patterns of several bacteria in different aspects of the environment like sewage is needed.

Methods

Design and Setting of Study

A laboratory-based cross-sectional quantitative study was designed. The study was conducted in Dharan sub-metropolitan city. It is a city in Koshi zone's Sunsari district of Province-1, Nepal with co-ordinates $26^0$49'0''N $87^0$17'0''E. The study was conducted from May 21, 2021, to October 29, 2021. Sewage effluents, directly from the municipal sewage channel, were taken as samples for the study. Non-probability convenience sampling method was followed for sample collection.

Sample Collection and Transportation

A total of 20 samples were taken from different sites of Dharan city. Samples were taken considering that it would cover almost every part of the city. Out of 20 samples, 6 samples (30%) were from the hospital area, and the remaining 14 samples (70%) were from the non-hospital area.

Samples were collected directly from the center of the sewage flow channel. For hospital sewages, samples were collected from the point where hospital sewage is connected to the municipal sewerage channel. A pre-sterilized sterile glass bottle was used to collect the sample from the flow channel with the help of a sampling rod. About 100 mL of sewage effluents were taken from one site. Each bottle was labeled with the date, sample location, and time of collection. Collected samples were transported to the laboratory immediately maintaining a cold chain in the ice-box to inhibit the growth of microorganisms. (Mahato et al., 2019)

Sample Processing and Inoculation

Samples were serially diluted in sterilized distilled water (SDW) up to tenfold. Eosin-methylene blue (EMB) agar plates and MacConkey (MAC) agar plates were prepared and solidified properly about 3–4 hours before inoculation. 5 plates of each media were prepared and labeled as $E10^{-2}$, $E10^{-4}$, $E10^{-5}$, $E10^{-6}$, and Econtrol on EMB plates and $M10^{-2}$, $M10^{-4}$, $M10^{-5}$, $M10^{-6,}$ and Mcontrol for MAC plates. From the serially diluted samples in the tube, 100 μL of the sample was pipetted out after mixing properly and inoculated on the agar plates using the spread-plate (lawn culture) method. Dilution 10^{-2}, 10^{-4}, 10^{-5}, and 10^{-6} were inoculated. The plates were incubated aerobically at 37^0C for 24 hours.

Isolation and Identification of E. coli **and** Klebsiella spp.

Following incubation colonies were isolated from the plate with well-isolated colonies. MAC was used for isolation of *Klebsiella spp*. and EMB was used for isolation of *E. coli*. (Mahato et al., 2019b) From MAC plates, well-isolated colonies which were showing characters of *Klebsiella spp.* were marked and streaked on Nutrient Agar (NA) plates for purification. Similarly, well-isolated colonies which were showing characters of *E. coli* in EMB plates were marked and isolated in NA plates (Anderson et al., 2019).

The isolated pure colonies on NA plates were Gram-stained and observe under a microscope for morphological characterization. After Gram staining, biochemical tests were performed. IMViC test, catalase test, oxidase test, motility test, H_2S production test, and TSI test were performed to confirm the identification of isolates as *E. coli* and *Klebsiella spp*(Rosa et al., 2016).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests of the isolates were performed by Kirby-Bauer disc diffusion method on Muller Hilton Agar (Hi-Media, India) according to CLSI 2017 guidelines (Patel & Clinical and Laboratory Standards Institute, 2017). Amoxicillin(10 µg), Aztreonam(30 µg), Azithromycin (15 µg), Ciprofloxacin (5 µg), Co-Trimoxazole (25 µg), Cefoxitin(30 µg), Ceftazidime(30 µg), Ceftriaxone(30 µg), Cefotaxime(30 µg), Imipenem(10 µg), and Nitrofurantoin (300 µg) were used for the AST. Following incubation, zone size was measured and compared with the AST chart provided by CLSI for *E. coli* and *Klebsiella spp*. for the tested antibiotic discs. All the isolated *E. coli* and *Klebsiella spp*. were tested for the production of ESBL and ABL enzymes.

Phenotypic Confirmation of ESBL Production

The combination disc method was used to phenotypically confirm the ESBL production by the isolated *E. coli* and *Klebsiella spp*. Two combination discs, Cefotaxime (CTX) 30μg vs. Cefotaxime + Clavulanic acid (CEC) 30/10 μg, and Ceftazidime (CAZ) 30μg vs. Ceftazidime + Clavulanic acid (CAC) 30/10 μg were used.

Those isolated *E. coli* and *Klebsiella* strains which showed an increase in zone size by $\geq$ 5 mm in combination discs (CAC and CEC) than those of CTX and CAZ discs alone were confirmed as ESBL

producers (Paterson & Bonomo, 2005).

Phenotypic Confirmation of ABL Production

The combination disc method was used for phenotypic confirmation of ABL production among isolated *E. coli* and *Klebsiella spp*. Combination disc Cefoxitin (CX) 30 μg vs. Cefoxitin + Cloxacillin (CC) 30/200 μg was used. Those isolated *E. coli* and *Klebsiella* strains which showed an increase in zone size by $\geq$ 4 mm in combination discs CC than that of CX alone was confirmed as ABL producers (Polsfuss et al., 2011).

Abbreviations

ESBL –	Extended Spectrum Beta-Lactamase
ABL –	AmpC Beta-Lactamase
BPKIHS-	BP Koirala Institute of Health Science
MSU –	Mid Stream Urine
MDR –	Multi Drug-Resistant
CLSI –	Clinical and Laboratory Standards Institute
ESBL - Eb-	Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae
pAmpC-Eb-	Plasmid Mediated AmpC Producing Enterobacteriaceae
SDW -	Sterile Distilled Water
MAC-	MacConkey
EMB-	Eosin Methylene Blue
CTX-	Cefotaxime
CEC-	Cefotaxime + Clavulanic acid
CAZ-	Ceftazidime
CAC-	Ceftazidime + Clavulanic acid
CX-	Cefoxitin
CC-	Cefoxitin + Cloxacillin

Declarations

Ethical Approval and Consent to Participate

Not applicable

Consent for Publication

Not applicable

Availability of Data and Materials

The datasets supporting the conclusion of this article are included within the article. The raw data will be available from the corresponding author on a reasonable request.

Competing Interests

The authors declare that they have no competing interests

Funding

Self-funded

Author's Contribution

PD and RR conceived the topic and designed the proposal. PD, RR, and MRS prepared the conceptual framework and methodology for experiments. LNC, PNC, PD, and MP performed the field works and experiments. RR and NK managed and analyzed the data. RR and PD wrote the paper. All authors read and approved the final manuscript.

Acknowledgments

We would like to express our sincere gratitude to Mr. Sagar Aryal for his technical support, guidance, and manuscript editing.

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Figures

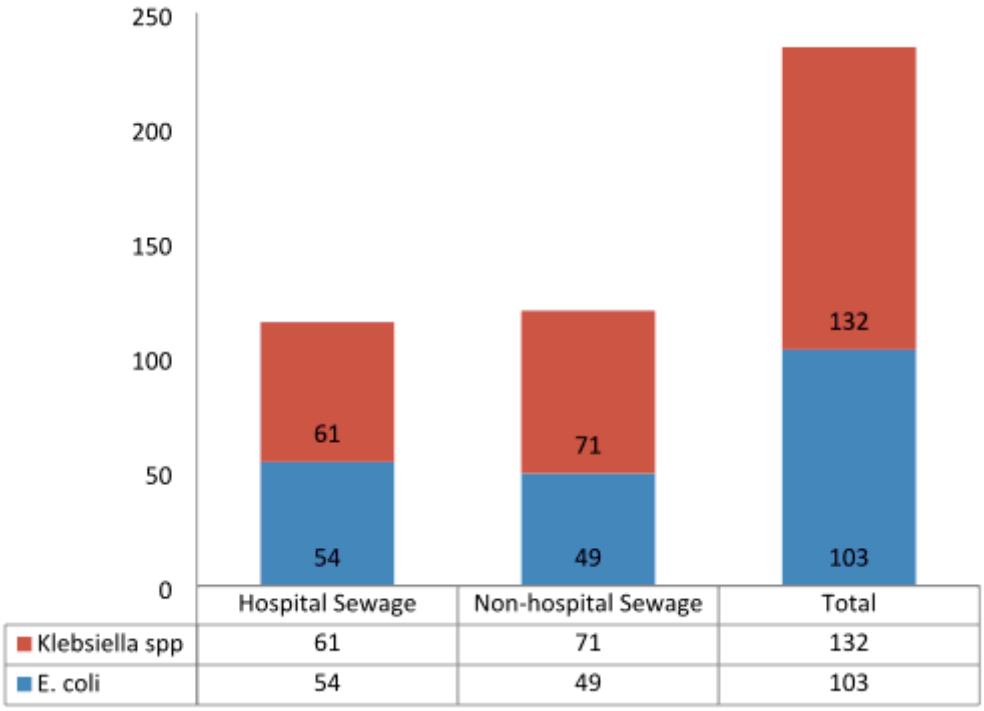
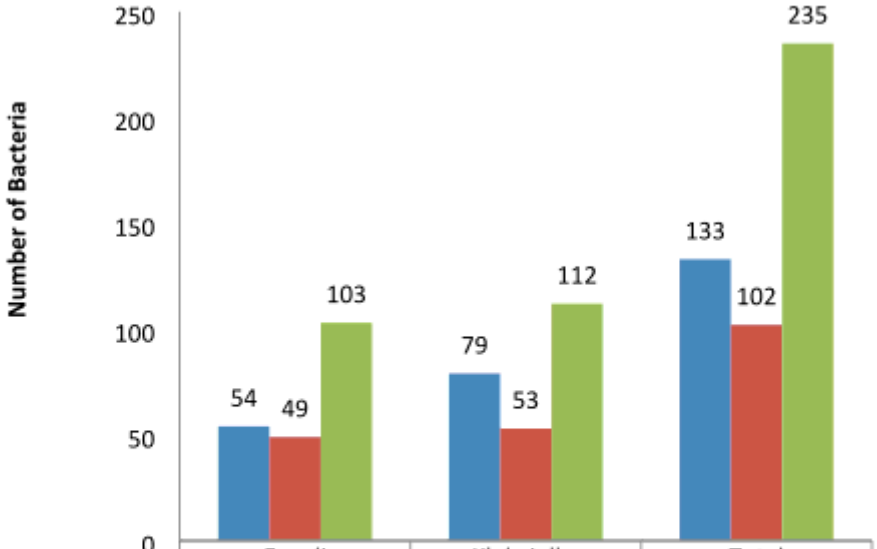


Figure 1

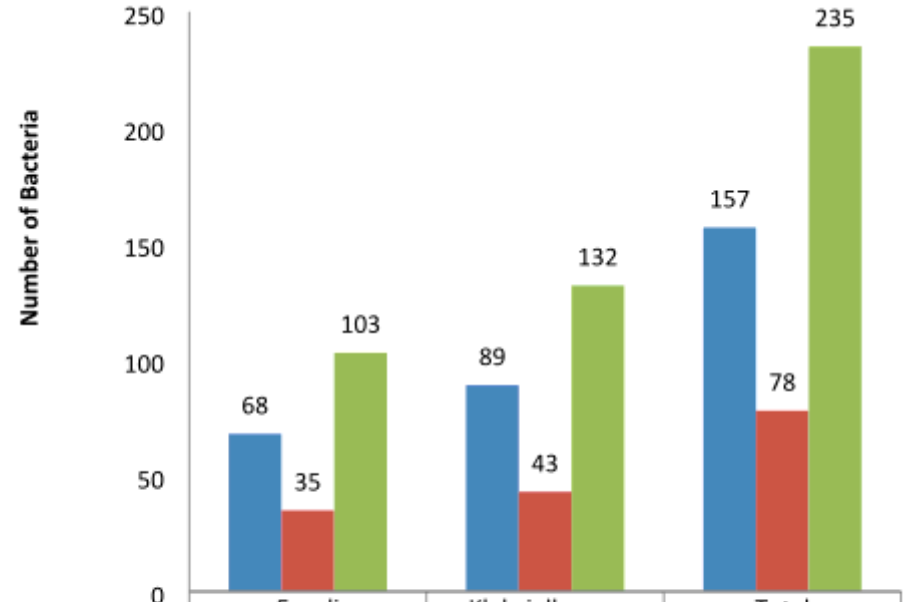
Proportion of isolated *E. coli* and *Klebsiella spp*.



	E. coli	Klebsiella	Total
ABL Producing	54	79	133
ABL Non-producing	49	53	102
Total	103	112	235

Figure 2

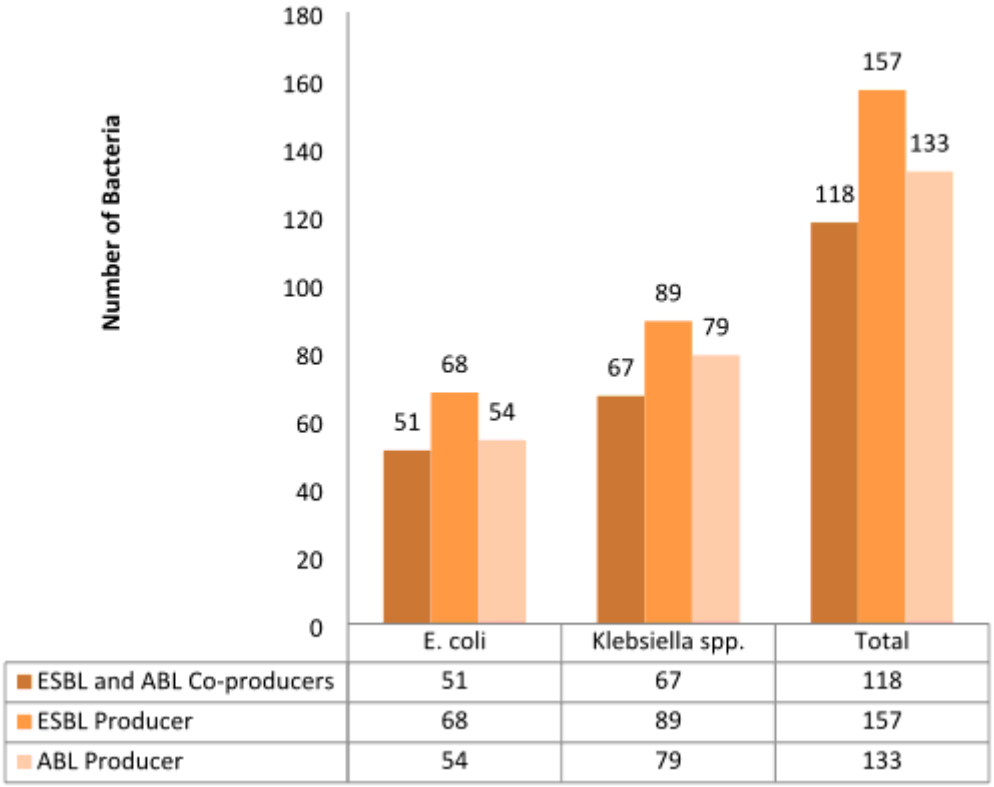
Distribution of ABL Producing *E. coli* and *Klebsiella spp*



	E. coli	Klebsiella spp.	Total
ESBL Producing	68	89	157
ESBL Non-Producing	35	43	78
Total	103	132	235

Figure 3

Distribution of ESBL Producing *E. coli* and *Klebsiella spp*



	E. coli	Klebsiella spp.	Total
ESBL and ABL Co-producers	51	67	118
ESBL Producer	68	89	157
ABL Producer	54	79	133

Figure 4

Distribution of ABL and ESBL Co-producing *E. coli* and *Klebsiella spp*