Extended-Spectrum Beta-Lactamase and Carbapenemase Producing Enterobacteriaceae among Patients with Gastrointestinal Complaints in North West Ethiopia

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Research

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Abstract

Background

Antimicrobial resistance is an increasing threat to health systems which leads to treatment failure, high treatment costs and increased mortality. Infections due to extended spectrum beta-lactamase (ESBL) and carbapenemase producing Enterobacteriaceae(CPE) impose a major global issues, because they are usually resistance to multiple antimicrobial agents. Data on the fecal ESBL producing Enterobacteriaceae (ESBL-PE) and CPE in developing countries including Ethiopia is limited mainly due to resource constraints. Thus, the aim of this study was to determine the prevalence of MDR, ESBL and CPE among patients with gastrointestinal complaints at the University of Gondar Comprehensive Specialized Hospital, Gondar; Northwest Ethiopia.

Materials and Methods

A Hospital based cross-sectional study was conducted among 384 patients with gastrointestinal complaints from January - April 2019 at the University of Gondar Comprehensive Specialized Hospital. A stool sample was aseptically collected and inoculated on MacConkey agar plate. After getting pure colonies, biochemical testing and antimicrobial susceptibility testing were done following standard microbiological techniques. ESBL production was screened by using ceftazidime and cefotaxime and confirmed using a combined disk diffusion test based on CLSI 2019 guidline. Carbapenemases were screened by meropenem disc and confirmed by modified carbapenem inactivation method. Data was checked, cleaned and entered using Epi-Info version 7.1 and transferred to SPSS version 20 for analysis. P-value <0.05 at 95% CI was considered as statistically significant.

Result

Out of the 384 study participants, 404 Enterobacteriaceae were isolated. Among these, 196 (48.5%) were MDR. The overall prevalence of fecal ESBL and CPE were 66(16.3%) and 4(1%) respectively. Of the total ESBL-PE, E.coli 41/66(62.1%) and K.pneumoniae 18/66(27.3%) were the most predominant isolates.

Conclusion

Finding high rate of MDR Enterobacteriaceae,ESBL-PE and CPE require strict infection control measures and careful selection of empirical therapy in the study area. Therefore, active surveillance with large sample size and better infection prevention and control is needed.

Background

Enterobacteriaceae are a group of Gram-negative, rod-shaped facultative anaerobe and their natural host is the human as well as animal intestine (1, 2). The human gastrointestinal tract is a reservoir for pathogens causing infections including urinary tract infections, nosocomial infections, skin and soft tissue infections. Bacterial translocation is the invasion of indigenous intestinal bacteria through the gut mucosa to normal sterile tissues and the internal organs (3, 4). Colonization of the gastrointestinal tract plays a key role in the epidemiology and clinical significance of extended spectrum beta lactamase (ESBL) and carbapenamase producing bacteria (5).

Extended spectrum beta lactamase-producing Enterobacteriaceae (ESBL-PE) have been reported worldwide since the early 1980s (6). In the past decade, there has been an alarming increase in antibiotic-resistant Enterobacteriaceae

producing ESBL due to overuse of broad-spectrum cephalosporins (7). Fecal ESBL-PE in the community was first reported in Spain and Poland in 2001 and 2002, respectively (8).

Extended spectrum beta lactamase producing Enterobacteriaceae have worldwide distributions with varying degree of prevalence in the community as well as hospitals (9, 10). Infections due to ESBL-PE and carbapenemase-producing Enterobacteriaceae (CPE) represent a major global health threat because they are usually resistant to multiple antimicrobial agents and lack of carbapenem drugs (11, 12). Although antimicrobial resistance is a global problem, the impact is higher in Sub-Saharan Africa due to limited available resources for healthcare infrastructure and wide irrational use of antimicrobial agents. From those who take antibiotics, more than one-third do not get prescriptions from a doctor and about a quarter obtain antibiotics from an informal dispenser (13, 14). Currently, infections due to ESBL-PE are concerning for many reasons including increased hospital costs, length of stay, treatment failure and mortality rates (15).

Carbapenemase producing Enterobacteriaceae are difficult to treat because of high levels of resistance to many antibiotics that break down all β-lactam agents including carbapenems and make it ineffective (16). The rise of ß-lactamase producing Gram-negative multidrug-resistant (MDR) organisms such as ESBL or carbapenemase-producing bacteria is the major particular concern (17–19).

The human gastrointestinal tract contains abandon normal flora and may act as a reservoir for other pathogens and these organisms that may contain the resistance gene may translocate through the gut mucosa to normal sterile tissues and the internal organs. As a result it may increase the resistance pattern in other sterile body site. However, data on the fecal ESBL and CPE is limited in developing countries especially in Ethiopia, particularly Gondar. Having the information on resistance pattern of normal commensals of human gastrointestinal tract are very important to encourage the prevention and control measures of the emergence of antimicrobial resistant strains.So this research intended to determine the magnitude of the intestinal ESBL and CPE at the University of Gondar Comprehensive Specialized Hospital.

Materials And Methods

Study design, area and period

A Hospital-based cross-sectional study was conducted at the University of Gondar Comprehensive specialized Hospital in Gondar town; Northwest Ethiopia, from January-April 2019. The town is located 737 km far from Addis Ababa, the capital city of Ethiopia and 180 km far from Bahir Dar, the regional capital. According to the central and statistical agency of Ethiopia report in 2015, the town has twelve sub city, twenty-two urban and eleven rural kebeles with a total projected population of 323,900. There are 8 health centers, 21 private clinics and one primary hospital in the town. The hospital provides health care service for more than 5 million people living in North, South, West Gondar Zones, as well as urban and rural kebeles surrounding the town.

Sample size and sampling technique

The sample size was determined using the single population proportion formula. By taking the prevalence of ESBLs and CPE infection that was conducted at Tikur Ambesa Specialized Hospital which showed 0.52 (13, 20), a total of 384 study participants were enrolled by using convenient sampling technique.

Laboratory Methods

Specimen collection and processing: The study participants were instructed to collect approximately 2 gram of diarrheal stool in to a clean, leak-proof container. The specimen of the study participants were collected at the University of Gondar Comprehensive specialized Hospital laboratory. Each stool sample was immediately transported to school of Bio medical and Laboratory Sciences, Medical Microbiology laboratory section using Cary-Blair transport media. Following an aseptic technique, a loop full of diarrhoeal sample was inoculated onto MacConkey agar (oxoid, **Code**: CM0115) and incubated aerobically at 37°C for 16-24 hours.

Identification

Preliminary identification: Preliminary identification of bacteria was based on their colony characteristics of the organisms.

Biochemical tests: were performed on isolated colonies for identification of Enterobacteriaceae based on their biochemical reaction. Biochemical tests includes triple sugar iron agar, indole test, citrate utilization test, urease production test, lysine decarboxylase test and motility test (21).

Drug susceptibility testing: Modified Kirby-Bauer disk diffusion technique using Muller Hinton agar (MHA) (Oxoid, UK) was used for antimicrobial susceptibility testing. Bacterial s uspension of three to five isolated colonies was done using 0.85% normal saline and the turbidity was adjusted at 0.5 % MacFarland standard. Using sterile cotton applicator stick, the suspension had been inoculated on MHA and left at room temperature for 3-5 minutes until it becomes dry. Then, diifferent antibiotic discs including ceftazidime (30µg) and cefotaxime (30 µg) were applied on inoculated MHA and incubated for 24 hr at 37°C. Ceftazidime (30µg) and cefotaxime (30 µg) discs were used for presumptive identification of ESBL production. The zones of inhibition was measured by a ruler and the results were interpreted as susceptible, intermediate and resistant using CLSI 2019 performance Standards for antimicrobial susceptibility testing interpretation table. Zone of inhibition ≤ 22mm for ceftazidime and ≤27mm for cefotaxime, was considered as potential ESBL producers (22).

Laboratory test for detection of ESBL and CPE

Confirmatory test for ESBL producer: The potential ESBL-PE was confirmed by combined disk method.Colony suspension of suspected ESBL-PE was inoculated on to MHA, then Ceftazidime (30µg) and Ceftazidime-Clavunalic acid (30/10 µg), Cefotaxime (30 µg) and Cefotaxime-Clavunalic acid (30/10 µg) disks were placed at 20 mm distance apart. If a ≥5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone, it was confirmed as ESBL-PE (22).

Screening test for CPE: Carbapenemase producing Enterobacteriaceae was screened by using Meropenem disks.Colony suspension of isolated bacteria was inoculated on to MHA, then Meropenem (10µg) disks were placed and incubated at 37^0c for 24 hrs. If the zone of inhibition is ≤ 19mm, it was considered as a potential CPE (22).

Confirmatory test for CPE: The suspected CPE is confirmed by Modified carbapenem inactivation method (mCIM). The isolated bacterial colony which was suspected for CPE was diluted with 2 ml of trypticase soya broth and meropenem (10µg) disk was immersed in the suspension; then incubate for 4 hours. A standard strain of meropenem susceptible *E.coli* ATCC 25922 was suspended in 0.85% normal saline and compared with MacFarland

standard (1:10 dilution) then inoculated the whole plate of MHA. After 4 hrs incubation, meropenem disk was removed from the test tube and placed on the MHA plate which was inoculated by *E.coli* ATCC 25922 meropenem sensitive strain and incubated at 37^{o}C for 18–24 hours. After incubation, if the zone of inhibition diameter between 6-15mm and 16-18mm with pinpoint colony, it was considered as carbapenem resistance Enterobacteriaceae (22).

Operational definitions

ESBL producers are bacteria that can produce the enzymes that confer resistance to most beta-lactam antibiotics (23).

MDR defined as resistance to three or more different classes of antibiotics (13).

Carbapenemases are beta-lactamase enzymes that inactivate almost all hydrolyzable beta-lactam antibiotics including the carbapenems (23).

Gastrointestinal tract complain is a discomfort in gastrointstinal tract with abdominal cramp, diarrhea, vomiting and distension of the abdomen (24).

Labolatory Quality control

All Medias were prepared according to the manufacturer's instruction and following standard operational procedure. All materials, equipment, and procedures was adequately controlled based on pre-analytical, analytical and post-analytical stages of quality assurance that were incorporated in standard operating procedures at the School of Bio-medical and Labolatory Sciences of Bio-Medical complex of Medical Microbiology section. Culture media was checked for sterility by incubating 5% batch of the media at 37^{o}c for 24 hours and performance test was checked by inoculating known control strains of *Escherichia coli* ATCC 25922 and *K. pneumoniae* ATCC® *700603* to confirm consistency of materials, methods and results. *K. pneumoniae* ATCC^{BAA}1705 and ATCC^{BAA} 1706 were used as a positive and negative quality control respectively for carbapenemase production.

Data analysis and interpretation

Data were collected, coded and entered in to EPI-Info version-7 to check completeness and clearance then transferred to SPSS version 20 for analysis.The characteristics of the study population were summarized using frequencies, percentages, mean and standard deviation and data was presented using tables.

Results

Socio-demographic characteristics of the study participants

Out of 384 study participants, 200(52.1%) were males while the remaining were females. The mean age of the study subjects were 30.76 ± SD 16.93. The highest frequency age group of the study participants were 16-30 years old 170(44.1%) and most of the study participants were urban residents 225(58.6%). Majority 310(80.7%) of the study participants were from outpatient department while the remaining were from inpatients (Table 1).

Table 1: Socio-demographic characteristics of patients with gastrointestinal complaints at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, January- April, 2019.

Demographic characteristics		Number (%)
Gender	Male	200 (52.1)
	Female	184 (47.9)
Age category (year)	≤5	28 (7.3)
	6-15	32 (8.3)
	16-30	170 (44.3)
	31-45	77 (20.1)
	46-60	52 (13.5)
	>61	25 (6.5)
Residence	Rural	159 (41.4)
	Urban	225 (58.6)
Occupation	Farmer	65 (16.9)
	Civil servant/employee	94 (24.5)
	Private	27 (7.0)
	House wife	48 (12.5)
	Student	100 (26.0)
	Other	50 (13.0)
Educational level	Illiterate	89 (23.1)
	Primary school	102 (26.6)
	Secondary school	46 (12.0)
	Higher education	119 (31)
	N/A	28 (7.3)
Marital status	Married	192 (50.0)
	Single	184 (47.9)
	Divorced	8 (2.1)
Department	Inpatient	74(22.9%)
	Out patient	310(80.7%)

Prevalence of Enterobacteriaceae

Out of 384 study participants, 404 Enterobacteriaceae were isolated in diarrhoea stool sample. From the total isolated Enterobacteriaceae, *E.coli* accounts the highest 219(54%) followed by *K.pneumoniae* 50(12%), *K. ozenae* 15(3.7%) , *Citrobacter species*14(3.5%), *Shigella* species 10(2.5%), *E.cloacae* 7(1.7%), *Proteus* species 6(1.5%), *Serratia species* 6(1.5%), *E.aerogenes* 1(0.2%), *S.typhi* 1(0.2%), and others Enterobacteriaceae 75(18.6%).

Multi Drug Resistant Patterns of Enterobacteriaceae

A total of twelve antibiotics such as Ampicillin, Ampicilin/Clavunalic acid, Gentamicin, Tobramycin, Tetracycline, Ciprofloxacin, Trimethoprim-sulfamethoxazole, Cefoxitin, Cefepime, Cefexime, cefuroxime and Ceftazidime or seven classes of antibiotics were used for antimicrobial Susceptibility Patterns of Enterobacteriaceae (Table 2). Of the total 404 isolated Enterobacteriaceae, 196(48.5%) were MDR that are resistant to three or more antibiotic classes. Among these, *E.coli* accounts the highest 118(60.2%), followed by *K.pneumoniae* 37(18.9%), *K.ozenae* 12(6.1%), *Citrobacter species* 10(5.7%), *Proteus* species 6(3.1%), *Serratia species* 5 (2.5 %.%) and *E.cloacae* 4(2.1%) (Table 3).

Table 3: Prevalence of MDR-Enteobacteriaceae from Patients with Gastrointestinal Complaints at the University of Gondar Comprehensive Specialized Hospital from January - April, 2019

Isolates	Degree of resistance				
	R3	R4	R5	R6	R7
E.coli (N=118)	30(25.4%)	34(28.8%)	29(24.5%)	20(16.9%)	5(4.2%)
K.pneumoniae (N=37)	12(32.4%)	11(29.2%)	9(24.3%)	3(8.1%)	2(5%)
K.ozenae (N=12)	6(50%)	3(25%)	2(16.6%)	1(8.3%)	---
Citrobacter species (N=10)	2(20%)	7(70%)	1(10%)	---	–
Proteus Vulgariss (N=6)	4(66.6%)	1(16.7%)	1(16.6%)	–	-
E.cloacae (N=4)	–	2(50%)	1(25%)	1(25%)	–
Salmonella species (N=1)	–	1(100%)	–	–	–
Shigella species (N=3)	2(66.7%)	1(33.3%)	–	–	–
Serratia species (N=5)	3(60%)	1(20%)	1(20%)	–	–
Total (N=196)	59(30.1%)	60(30.6%)	44(22.4%)	25(12.7%)	7(3.5%)

Note: R3, R4, R5, R6 and R7: Resistance to 3, 4, 5, 6, and 7 classes of antibiotics respectively.

Prevalence of ESBL and Carbapenemase Producing Enterobacteriaceae

Based on the CLSI standard only *E. coli, K.pneumoniae, K.ozenae and Proteus vulgaris* were screened using Ceftazidime (30µg) and cefotaxime (30 µg) discs and tested for ESBL production. Of the total of 404 isolated Enterobacteriaceae, 106(26.3%) were screened positive and 66/106(62.2%) were confirmed ESBL-PE. Of these, *E. coli* accouns 41 (62.1%) followed by *K.pneumoniae* 18(27.3%), *K.ozenae* 4 (6%) and *Proteus vulgaris* 3(4.5%) (Figure 1). As per CLSI 2019 Guildline, other Enterobacteriaceae groups were excluded from ESBL detection because they have no breakpoint in the guidelines.

A total of 105 Enterobacteriaceae (66 were confirmed ESBL-PE and the rest 39 were other Enterobacteriaceae) were screened for carbapenemase production by using Meropenem disk. Among these, 4(6%) such as *[Citrobacter species* (2), *E.coli* and *P.vulgaris* each accounts 1] were presumptive carbapenemase producer. These four presumptive CPE were confirmed by modified carbapenem inactivation method (mCIM) and all 4/4(100%) were CPE. From the total CPE, *Citrobacter species* accounted 2 (50%), *E.coli* 1(25%) and *P.vulgaris* 1 (25%) (Table 4).

Table 4**:** Distribution of ESBL and Carbapenemase producing Enterobacteriaceae from Patients with Gastrointestinal Complaints at the University of Gondar Comprehensive Specialized Hospital from January to April, 2019.

Bacterial Isolates	ESBL Screening N(%)		Confirmed ESBL N(%)		CPE Screening N(%)		Confirmed CPE N(%)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
E.coli	69 (65%)	150	41(62%)	28(70%)	1(25%)	40(40%)	1(25%)	0
K.pneumoniae	29 (27%)	21	18(27%)	11(28%)	0	18(18%)	0	0
K.ozenae	4 (4%)	11	4(6%)	0	0	4(4%)	0	0
Citrobacter species	N/T	N/T	N/T	N/T	2(50%)	12(12%)	2 (50%)	0
Proteus Vulgariss	4 (4%)	2	3(5%)	1(3%)	1(25%)	2(2%)	1 (25%)	0
E.cloacae	N/T	N/T	N/T	N/T	0	7(7%)	0	0
S. typhi	N/T	N/T	N/T	N/T	0	1(1%)	-	-
Shigella spp.	N/T	N/T	N/T	N/T	0	10(10%)	-	-
Serratia species	N/T	N/T	N/T	N/T	0	6(6%)	-	-
E.aerogenes	N/T	N/T	N/T	N/T	0	1(1%)	-	-
Total	106	184	66	40	4	101	4	0

N/T= Not tested

Discussion

Even if the human GI tract contains full of commensal, they may act as a reservoir for pathogens and may translocate in to sterile site through genetic elements (plasmids). As a result it may increase the resistance pattern. So conducting a research on commensal is very crucial for identifying the resistance strains. In the current study from the total 384 study participants, 404 Enterobacteriaceae were isolated. Among these, *E.coli* accounts the highest 219(54%) followed by *K.pneumoniae* 50(12%), *K. ozenae* 15(3.7%) and *Citrobacter* 14(3.5%).The result was lower than a study conducted in Addis abeba, Ethiopia which shows *E. coli* (79.7%) was the highest followed by *k.pneumoniae* (19.7%) (13); in Libya *E.coli* (55%), *K.pneumoniae* (28.8%) (25); United Arab Emirates *E.coli* (63.8%), *K.pneumoniae* (34.6%) (26). This difference may be due to the technique that we used, sample size, geographical distribusion and the study population.

In the present study, the overall prevalence of ESBL was 66/404(16.3%) which is concordant with a report in France (17.7%) (27). However, it was lower than a report in Tikur Anbesa Specialized Hospital, Addis Abeba (52%) (13), Madagaskar 49% (28), Egypt (65%) (12), Mozambic University (20%) (18), Guinea-Bissau (32.6%) (29), Morocco (42.8%) (30), Tanzania (34.3%) (31), Beirut (24.5%) (32), Southeast Asia (50.7%) (6), Venezuela (34.6%) (33), Turkey (30%) (34), Sweeden 35% (35), Korea (28%) (36) and Argentina 18.9% (37). This variation may be due to the diferrence in diagnostic method, study population and geographical location. In contrast, the current finding was higher than a study conducted in Libiya (13.4%) (25), Japan 6.2% (38), Amsterdam (8.6%) (10), France 4.6% (39), Soudi Arabia 91(12%) (40), Netherland 73(10%) (41), Norway 15.8% (11), Swetherland (5.8%) (42). This variation may be due to the antibiotic practice used in the population, geographical location, and poor personal and environmental hygienic practices.

Our finding showed that 41/66 (62%) *E.coli* were ESBL positive which is inline with a report in Mozambic University (62%) (18). But lower than a study done in Tikur Ambesa Specialized Hospital, Addis Abeba (70%) (13), Norway (86%) (11), Saudi Arabia (95.6%) (37), France (89%) (39), Japan (78.5%) (38), Southeast Asia (97%) (6). However, it

was higher than a study conducted in Madakascar 32% (28), Guinea-Bissau (47%) (29), Morocco 48.5% (30), New York (60%) (26). This discrepancy of isolation may be due to geographical location, poor hygienic practice, irrational use of antibiotics, and study populations.

In this study, the over all prevalence of CPE was 4/404(1%), but the finding is not concordant with other studies. As a result, the present study is lower than a study conducted in Tikur Ambesa Specialized Hospital, Addis Abebea (2%) (13), Egypt (2%) (43), Morocco (13%) (30), Uganda 10% (44), India (6.6%) (45), Mexico (16.6%) (46). However, our study reported higher prevalence than a study done in Mozambic (0.8%) (18), Norway (0%) (11), Korea (0.3%) (36), Asia 0.6% (47). This discrepancy of isolation may be due to geographical location, poor hygienic practice, inappropriate use of antibiotics, cross boarder of patients with other countries of high prevalence, sample size, methodological variability could bring variation in the prevalence of CPE.

In this study, the overall prevalence of MDR-Enterobacteriaceae (MDR-E) was 196(48.5%) which is similar with a study done in Norway 48% (11). However, our result is lower than a study done in Mozambic University 88% (18) and Switherland 51% (42). But it was higher than a study conducted in Tikur Ambesa Specialized Hospital Addis Abeba 43% (13), Madagaskar 41.9% (28), Morocco 42.8% (30), India 12.4% (45). This discrepancy may be due to irrational use of antibiotic, poor personal and environmental hygienic practis in the study area and also scarcity of proper diagnostic tools and also it may be consumption of animal product that takes antibiotics for growth promotion and teatment purpose, increased trend of MDR strains with time, differences in study population, failure of patient adherence to their medication, and unavailability of guidelines for the selection of antibiotics.

Limitation of the study

This study was conducted in the hospital setting in a small sample size. This study also did not include other important pathogens responsible for gastro intersinal complain due to lack of laboratory facility.

Conclusion

Multi drug resistant, extended spectrum beta lactamase and carbapenemase producing Enterobacteriaceae were higher in the gastrointestinal tract infections. *E.coli* was the most predominant ESBL-PE followed by *K.pneumoniae*.

Finding high rate of drug resistance in Gram-negative bacteria require strict infection control measures and careful selection of empirical therapy in the study area. Even if many Enterobacteriaceae are human fecal normal flora, they carry resistance gene and act as a reservoir. If MDR-Enterobacteriaceae is isolated ESBL and CPE should be screened to improve the infection prevention practice. Active surveillance with large sample size will also be performed to know the high prevalence of ESBL and CPE in gastrointestinal tract infections.

List Of Abbreviations

ATCC: American Type Culture Collection; AMR: Anti Microbial Resistance; CLSI: Clinical and Laboratory Standard Institute; CPE: Carbapenemase Producing Enterobacteriaceae; CRE: Carbapenem Resistance Enterobacteriaceae; ESBL: Extended Spectrum Beta-Lactamase; ESBL-PE: Extended Spectrum Beta-Lactamase-Producing Enterobacteriaceae; GIT: Gastro-Intestinal Tract; ICU: Intensive Care Unit; mCIM: Modified Carbapenem Inactivation Method; MDR: ulti Drug Resistance; OPD: Out Patient Department; QC: Quality control.

Declarations

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Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Authors' contributions

MW collected the data.MW and AG wrote the manuscript. AG, FM and MG gave valuable suggestions of the manuscript and revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was conducted after obtaining ethical clearance from ethical review committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences University of Gondar. Legal permission letter was also obtained from hospital clinical directors prior to data collection. Only those who were volunter to participate in the study was asked to give samples and soco-demomgrapic data. Written Consent was obtained from study participnts and guardians. In addition to consent from the guardians, an assent was also asked from under 18 years old patients. Information obtained from each participants was kept confidential.

Consent for publication

Not applicable

Cempeting interests

The authors declare that they have no competing interests.

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Figures

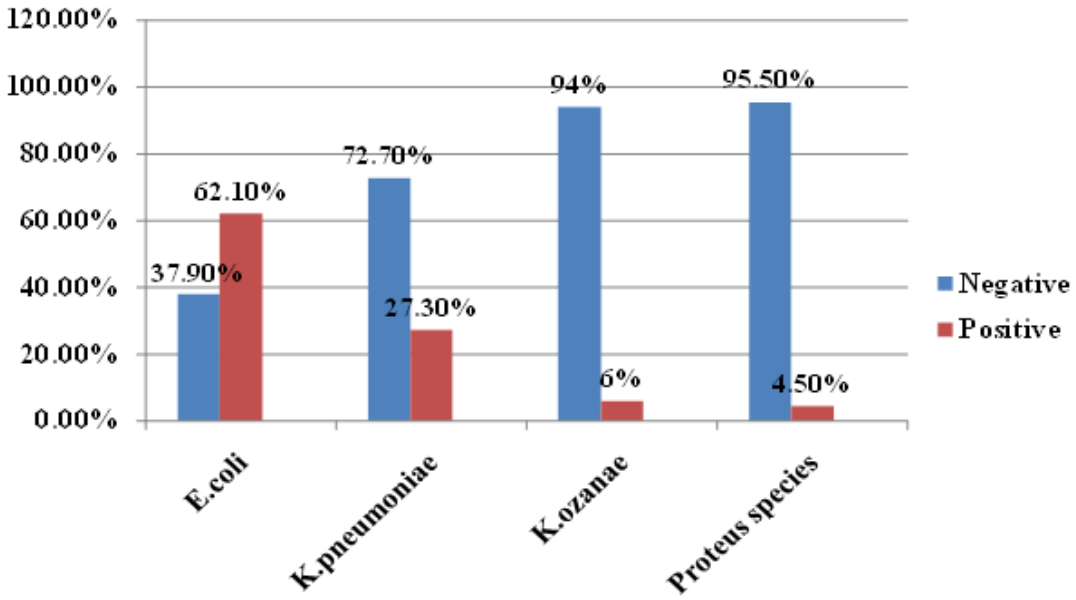

Figure 1

Distribution of ESBL-PE from Patients with Gastrointestinal Complaints at the University of Gondar Comprehensive Specialized Hospital from January - June, 2019