

Extended Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* and *Klebsiella* species in pediatric patients visiting International Friendship Children's Hospital, Kathmandu, Nepal

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Research

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Abstract

Background Emergence of antibiotic resistance among pathogenic strains has spread due to production of β -lactamases, which can lead to failure of empirical therapy in clinical settings. Inappropriate use of antibiotics, particularly third generation cephalosporins has contributed to the development of antimicrobial resistance (AMR). This study aims to determine the prevalence of Extended Spectrum β -Lactamase (ESBL) production in *E. coli* and *Klebsiella* species isolated from various clinical samples.

Methods This cross-sectional study was conducted at International Friendship Children's Hospital, Kathmandu, Nepal from August 2017 to January 2018. Various clinical samples that included urine, pus, Cerebro-Spinal Fluid (CSF), body fluids, wound swab, endotracheal tip, catheter tip and blood were processed for culture. Following sufficient incubation, isolates were identified by colony morphology, gram staining and necessary biochemical tests. Identified bacterial isolates were then tested for antibiotic susceptibility test by modified Kirby Bauer disc diffusion method, and were subjected to Extended Spectrum Beta Lactamase (ESBL) screening by using 30 μ g cefotaxime and ceftazidime. ESBL production was confirmed by combination disc method.

Results From a total of 103 non-duplicated clinical isolates, *E. coli* (n=79), *Klebsiella pneumoniae* (n=18) and *K. oxytoca* (n=6) were isolated from different clinical specimens. Majority (62.1%; 64/103) exhibited Multi-Drug Resistance (MDR) and 28.2% (29/103) were ESBL producers. All of ESBL producing isolates were resistant towards ampicillin, cefotaxime, ceftriaxone, ceftazidime. Most ESBL producers were found to be susceptible towards imipenem (89.7%; 26/29), nitrofurantoin (82.8%; 24/29), piperacillin/tazobactam (79.3%; 23/29), and Amikacin (72.4%; 21/29).

Conclusions High prevalence of multi-drug resistant ESBL organisms found in this study warrants restricting empirical treatment of the bacterial infection. Identification of ESBL producers in routine treatment of infectious diseases can reduce unnecessary and inappropriate antimicrobial use and can reduce the preventable morbidity and mortality.

Introduction

Escherichia coli (*E. coli*) and *Klebsiella* species are the most common causative pathogens for most of the infections especially in countries with poor health care system [1]. *E. coli* is a normal flora of human and animal gut but can also be found in water, soil and vegetation [2]. *Klebsiella* species are considered as major opportunistic pathogens that can cause nosocomial infections. *Klebsiella pneumoniae* is an important cause of human infections among all *Klebsiella* species followed by *K. oxytoca*, *K. ozaenae* and *K. rhinoscleromatis* [3].

E. coli is one of the most frequent causative agents of several common bacterial infections such as gastroenteritis, Urinary Tract Infection (UTI), septicemia, and neonatal meningitis. *Klebsiella* species are the other common organisms that can cause pneumonia, UTI, bacteremia, septicemia and soft tissue infections [3, 4].

Commonly used antimicrobial agents against these pathogens are tetracycline, beta-lactams, fluoroquinolones, aminoglycosides and cotrimoxazole. However, antimicrobial resistance (AMR) among Enterobacteriaceae has increased dramatically in recent years limiting the therapeutic options. Isolates that are not susceptible to at least three or more groups of antimicrobials are known as Multi Drug Resistant (MDR) organisms [5]. The Infectious Disease Society of America has listed *E. coli* and *Klebsiella* species as two out of six pathogens for which new drugs are urgently needed in order to combat their growing resistance [6]. The mechanism of resistance to these antibiotic in these organisms is primarily developed through the production of β -lactamase enzymes, that includes extended spectrum β -lactamase (ESBL), AmpC β -lactamase (ABL), and carbapenemase which are responsible for resistance to β -lactam antibiotics such as penicillins, cephamycins and carbapenems [7, 8].

Beta- lactams comprise the largest group of antibacterials that includes penicillins, cephalosporins, carbapenems, and monobactams; and are the most common drugs against infectious diseases. Resistance to beta-lactams is primarily because of β -lactamase enzymes produced by bacteria that hydrolyze the beta- lactam ring, thereby inactivating the drug [9]. Extended-spectrum β -lactamases (ESBLs) are class A β -lactamases, a rapidly evolving group of β -lactamases with the ability to hydrolyze and cause resistance to the oxy-imino cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) and monobactams (aztreonam) [10]. These plasmid-mediated enzymes are primarily produced by Enterobacteriaceae family of gram-negative organisms, in particular, *Klebsiella pneumoniae* and *E. coli*; and also by some non-fermentative gram-negative organisms such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [11, 12].

Antibiotic resistant bacteria are emerging worldwide and continue to pose significant threat to the current options for treatment of common infections in community and hospital settings [13]. ESBL producing *E. coli* and *Klebsiella pneumoniae* are the predominant cause of childhood infections and presents significant challenges [14] such as development of adverse outcomes [15], treatment failure due to multidrug resistance, and high morbidity and mortality [16]. Empirical and symptomatic (without a diagnosis) use of antibiotics in resource poor settings is responsible for higher incidence of antibiotic resistance among bacteria [17].

Several studies in the past have investigated the prevalence of ESBL organisms among inpatients mostly focused in adult patients [7, 18, 19]. Studies have shown the varying prevalence of ESBL organisms, for instance, the prevalence was 27.7% in Pokhara [7], 18% in Kathmandu [20], 43% in pediatric hospital in Kathmandu [21]. Another study reported 35.9% ESBL in *E. coil* isolates among outpatients at tertiary care hospital in Kathmandu [18]. However, similar study from Lalitpur district reported 6.8% ESBL producing isolates [19]. Studies have shown the wide range in prevalence of ESBLs (10 to 43%) in different hospitals/settings from various samples.

Although it is deemed to be essential to have a routine diagnosis and monitoring of ESBL producing clinical isolates in clinical laboratories, ESBL screening as a routine test has not yet been practiced in Nepal [22]. In addition, very few studies have reported on ESBL producing clinical isolates from pediatric

patients in Nepal. Only one study in the past has reported ESBLs (prevalence: 38.9%) from urine samples in pediatric patients from a tertiary teaching hospital in Kathmandu [23]. Expanding and building on the previous research, this study focused to isolate both *E. coli* and *Klebsiella* spp. from wider and larger number of clinical specimens from pediatric patients. The main objectives of this study was to explore the prevalence of ESBL producing organisms including the resistance types among pediatric patients attending a tertiary care pediatric hospital at Kathmandu.

Methods

Study design, area and sample population

This was a cross-sectional study conducted at International Friendship Children's Hospital, Maharajgunj, Kathmandu, Nepal during August 2017 to January 2018. The study population comprised children below 15 years attending the hospital.

The specimens were collected adhering to a standard protocol from pediatric patients below or equal to 15 years with demographic details. Children with incomplete demographic information, and those who were suffering from co-morbid conditions such as chronic diseases, malnutrition, and other abnormalities based on the clinical assessment were excluded. Patients who were undergoing antibiotic treatment were also excluded.

A total of 1,443 different samples that included urine, pus, wound swab, endotracheal tip, catheter tip, and blood were collected and processed by standard microbiological methods [24].

Sample collection and transport

Special measures were taken to collect the urine samples from children who were not able to use toilet on their own. An adhesive, sealed, sterile collection bag was placed underneath the genitalia to collect urine sample. Toilet trained children were requested to collect mid-stream urine assisted by their parents in a sterile, dry, wide-necked and leak-proof container. In either condition, cleansing of genitalia was done to reduce contamination.

In the case of infected wounds, in addition to wound swab, pus was aspirated in syringe by trained medical personnel. In case pus was not discharging, cotton swab was gently rolled over the surface of the wound approximately five times, focusing on areas where there was evidence of pus or inflamed tissue. Two swabs were taken from each patient, one for culture and another for direct gram staining.

About 2 ml of blood from children was withdrawn and dispensed into sterile screw capped culture bottles containing BHI (Brain Heart Infusion) broth. Specimens were collected from other sources such as endotracheal and catheter tips by trained medical personnel. The collected samples were labeled properly and were immediately delivered to a laboratory for further processing. When immediate delivery was not possible, the specimens were refrigerated at 4–6 °C [25].

Laboratory examinations of samples

Microscopic examination

Smears of specimens such as pus were gram stained and observed through microscope for the presence of bacteria and pus cells.

Culture

For processing of each sample, microbiological protocols were followed according to standard microbiological guidelines [24, 26].

Urine sample: Using a sterile calibrated loop, urine sample was inoculated on MacConkey agar (MA) and Blood agar (BA), then incubated aerobically at 37°C for 24 hours. Colony count was made and positive result was considered for plates showing more than or equal to 10⁵ colony forming units (CFU)/ml of urine.

Blood sample: Blood sample was incubated on brain heart infusion (BHI) broth for seven days at 37°C. Bottles showing turbidity during the period were sub-cultured aerobically in MA and BA at 37°C for 24–48 hour.

Pus, wound swab, CSF, body fluids: These specimens were inoculated into MA and BA plate and incubated at 37°C overnight.

Other specimens: Endotracheal and catheter tips were first incubated on BHI broth at 37°C for 24 hours and sub-cultured on MA and BA plates and incubated at 37°C overnight.

Identification of *E. coli* and *Klebsiella* species

Presumptive identification of *E. coli* and *Klebsiella* species was done on the basis of colony colour and Gram's staining morphology. Then obtained pure cultures of isolates, were assessed for biochemical tests for confirmation [24, 26].

Antibiotic Susceptibility Testing (AST)

All identified isolates of *E. coli* and *Klebsiella* species were treated for susceptibility testing against ampicillin (10µg), gentamicin (10µg), amikacin (30µg), aztreonam (30µg), cefoxitin (30µg), ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg) ciprofloxacin (5µg), imipenem (10µg), piperacillin/tazobactam (100/10µg), nitrofurantoin (300µg), and cefepime (30µg) (Hi-Media India Pvt. Ltd) following Kirby-Bauer method on Mueller-Hinton Agar (Hi-media India Pvt. Ltd.). Results were

interpreted based on the CLSI 2016 guidelines [27]. Those isolates which were not susceptible (either resistant or intermediate) to three or more antibiotics classes were considered as multi-drug resistant [5].

Screening and confirmation of ESBL producers

Bacterial isolates were screened for possible ESBL production using ceftazidime (30µg), cefotaxime (30µg), ceftriaxone (30µg), and aztreonam (30µg). Results were interpreted using CLSI guidelines. The screening positive bacterial isolates were subjected to combination disk diffusion test for confirmation of ESBL production that involved using disks consisting of ceftazidime (30µg), and ceftazidime plus clavulanic acid (30/10µg). Result was interpreted by comparing the zone of inhibition [27].

Quality control

Each batch of media and reagents were subjected to sterility and performance testing. During antibiotic susceptibility test, quality control was done using the control strains of *E. coli* ATCC 25922. Strict adherence to aseptic conditions and laboratory safety was adopted during each steps of examination.

Data management and statistical analysis

Data were entered and analyzed by using IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY: IBM Corp. Descriptive and inferential statistics were analyzed.

Results

Prevalence of bacterial isolates

A total of 1,443 different clinical specimens were processed during the study, out of which 299 (20.7%; 299/1443) samples showed bacterial growth. Among the isolates, (26.4%; 79/299), (6.0%; 18/299), (2%; 6/2996) were identified as *E. coli*, *Klebsiella pneumoniae* and *K. oxytoca* respectively and remaining 65.6% (196/299) were other bacterial isolates. *E. coli* was predominant bacteria isolated from urine samples (86.0%; 68/79) followed by pus/wound pus samples (8.8%; 7/79). *K. pneumoniae* (77.8%; 14/18) and *K. oxytoca* (83.3%; 5/6) were mostly isolated from urine sample (Table 1).

Antibiotic susceptibility pattern of bacterial isolates

Among 103 bacterial isolates, 90.3% (n = 93) were found to be susceptible to imipenem followed by piperacillin/tazobactam (88.3%; n = 91), nitrofurantoin (85.5%; n = 88) and amikacin (82.5%; n = 85). Majority of *E. coli* isolates (92.4%; 73/79) were found to be susceptible to Imipenem, followed by Nitrofurantoin (91.2%; 72/79). Similarly, 88.9% (16/18) of *K. pneumoniae* were found to be susceptible to amikacin. *K. oxytoca* were found to be 100% (6/6) susceptible to gentamicin, piperacillin/tazobactam,

imipenem. The antibiotics: ampicillin, cefotaxime, ceftriaxone, ceftazidime, ciprofloxacin were found to be most ineffective against *E. coli*, *K. pneumoniae* and *K. oxytoca* isolates (Table 2).

Multi Drugs Resistance (MDR) Profile in bacterial isolate

Bacterial isolates showing resistant to three or more antibiotic classes were considered as MDR isolates. Among the total of 103 bacterial isolates, 62.1% (64/103) were found to be MDR, the highest MDR strains were detected in *K. pneumoniae* (88.9%; 16/18), followed by *E. coli* (57%; 44/79), and *K. oxytoca* (50%; 3/6) (Figure 1).

ESBL production among *E. coli* and *Klebsiella* species

Among the total *E. coli* and *Klebsiella* species, 51.5% (53/103) were suspected as ESBL producer on primary screening test; out of which 28.2% (29/103) were confirmed as ESBL producer by combination disk diffusion method. The highest percentage of ESBL production was found among *K. pneumoniae* (33.3%; 6/18) followed by *E. coli* (27.9%; 22/79) and *K. oxytoca* (16.7%; 1/6) (Table 3).

Distribution of ESBL producers according to different age of patient

Among the 103 isolates, 77.7% (n = 80) were isolated from children \leq 5 years age followed by 6–10 years age group children, 15.5% (n = 16). Of 103 bacterial isolates, 28.1% (n = 29) were ESBL producers and majority of them 82.8% (n = 24) were isolated from children \leq 5 years age. There was no association between ESBL producers and age of patients ($p < 0.05$) (Table 4).

Distribution of ESBL producers in different wards of hospitals

Of 29 isolates of ESBL producers, 51.7% (n = 15) were from inpatients whereas 48.3% (n = 14) were from Out Patient Department (OPD). There was no significant association between ESBL production and type of the patients ($p > 0.05$) (Table 5).

Antibiotic susceptible pattern of ESBL producers

All of ESBL producers' isolates were found to be resistant towards cefotaxime, ceftriaxone, ceftazidime, and ampicillin. Most ESBL producers were found to be susceptible towards imipenem (89.7%; 26/29), nitrofurantoin (82.8%; 24/29), piperacillin/tazobactam (79.3%; 23/29), and Amikacin (72.4%; 21/29) (Figure 2).

Discussion

Overall findings

This study found the high prevalence (>60%) of Multidrug Resistance (MDR) bacteria in clinical specimens isolated from the tertiary care hospital of children in Kathmandu valley. Among MDR isolates, half of the isolates were ESBL producers. Majority of ESBL producer-isolates were found to be resistant towards cefotaxime, ceftriaxone, ceftazidime, and ampicillin. Similar findings were reported in previous studies in different clinical settings of Nepal [18, 23, 28, 29].

Majority (>80%) of bacterial isolates were isolated from urine sample, thus re-affirming urinary tract infection (UTI) to be the most common cause of febrile illnesses in children requiring antimicrobial treatment [30]. Worldwide, an estimated 8% of girls and 2% of boys experience at least one episode of UTI by the age of seven years and recurrence occurs in 12–30% of them in a year. Pediatric UTI in many instances remain under-diagnosed because of the absence of specific symptoms and signs, particularly because of their inability to explain adequately [31, 32]. Majority of *E. coli* (>80%) were isolated from the urine samples and predominance of *E. coli* in urine has been reported from studies conducted in different hospitals of Nepal [7, 21, 23, 29]. The predominance of *E. coli* followed by *K. pneumonia* and *K. oxytoca* in urine samples was also reported in several previous studies conducted in Nepal [33–35].

Majority of the isolates (>80%) in this study were found susceptible to imipenem, piperacillin/tazobactam and amikacin. The high efficacy of amikacin and imipenem against *E. coli* and *Klebsiella* were also reported from studies conducted in Chitwan [33], and Lumbini [35], Nepal. The findings were also in line with a study from Manipal Teaching Hospital, Pokhara [7]. Because of better pharmacokinetics for treatment of UTI than other infections, nitrofurantoin was only tested for urinary isolates [36]. Nitrofurantoin was highly effective against *E. coli* (91.2%) followed by *K. pneumoniae* (68.8%) and *K. oxytoca* (66.7%) which is similar to the study reported from a tertiary setting [37].

Majority of isolates (77.5%) were resistant to ampicillin including more than half of the cephalosporin group of antibiotics. Similar findings were observed in a tertiary hospital in Pokhara, Nepal [7]. This type of resistance could be due to production of several beta lactamase enzymes. Since ampicillin is the first line β -lactam drug for Enterobacteriaceae, it can be easily hydrolyzed by β -lactamase enzymes. Resistance to fluoroquinolones is due to mutation at the target site i.e. *gyrA* (gyrase subunit gene) and *parC* (topoisomerase subunit gene) and efflux [38].

Antimicrobial resistance (AMR) including MDR is a global problem, and its burden varies between the regions, however, low- and middle-income countries share a disproportionate burden due to multitude of factors embedded in the characteristics of the health system, policy and the practice [39]. Moreover, MDR pathogens are more common in hospital-settings and are mostly of nosocomial origin which are often difficult to treat [40]. MDR pose a major threat in the management of uropathogens [41–43]. More than two-thirds of the isolates in this study were MDR, mostly being *E. coli*, *K. pneumoniae* and *K. oxytoca*. High prevalence of MDR strains have been reported consistently from past studies from within

Kathmandu [17, 18, 23, 40] and outside [15, 20, 22]. Bacterial resistance to β -lactam antibiotics has risen dramatically, with significant contribution by extended spectrum β -lactamase (ESBL) [44].

E. coli and *K. pneumoniae* are major ESBL producers posing serious threat to the treatment regimen [37]. ESBL enzymes are becoming increasingly expressed by many strains of pathogenic bacteria presenting diagnostic challenges to the clinical microbiology laboratories [45]. The factors leading to the high prevalence of the ESBL producers could be due to indwelling catheters, invasive procedures, excessive use of cephalosporins and severity of the illness. In addition, higher prevalence of ESBL isolates among children might be due to poor nutritional status, low birth weight, and weak immune system [46]. The occurrence of ESBLs among clinical isolates varies globally and the epidemiological burden has been shifting rapidly in low and middle income settings in recent years [47, 48].

The highest bacterial isolates were found in children less than 5 years age including the prevalence of ESBL organisms which was above 80%. The reason for this may be due to the immunological status of the children below 5 years of age. In general, the younger children are more vulnerable and get infected easily than older children. The marginal higher prevalence of bacterial growth in inpatients may be due to the nosocomial infections as well. Nosocomial infections are attributed to the longer hospital stay, prolonged stay in ICU, extensive use of invasive medical devices and over consumption of antibiotic among inpatients [49, 50].

Most ESBL organisms were susceptible to imipenem, piperacillin/tazobactam, amikacin and nitrofurantoin. However, ESBL producers were resistant to ampicillin, and cephalosporin group of antibiotics. These findings are consistent with similar studies reported from Nepal [7, 37, 47, 51]. The resistance to fluoroquinolone may have been the result of alterations in target enzymes (DNA gyrase and topoisomerase IV) including change in drug entry and efflux [49]. The high proportions of resistance to third generation cephalosporins reported for *E. coli* and *Klebsiella pneumoniae* means that treatment of severe infections are likely to be caused by these bacteria in many settings and must rely on carbapenems, the last resort to treat severe community and hospital acquired infections [52].

Increasing spectrum of ESBL drug resistant bacterial isolates can cause major problems for physicians in choosing from the available therapeutic options, if these organisms are not routinely isolated. Reporting of ESBL producing isolates from clinical samples is thus critical for the clinicians to select appropriate antibiotics for the treatment including to take proper precaution to prevent the spread of these resistant organisms to other patients.

Strengths and limitations

This study will be a useful reference for future studies, to explore and expand on the wider prevalence of ESBL organisms in clinical and non-clinical settings. Since our study was based on phenotypic detection of AMR and ESBL production that excluded identification and characterization of wide sorts of lactamases and pathogenic strains, genotypic characterization is recommended in future studies.

Implications for antimicrobial resistance and its control

This study has identified one of the major determinants of burgeoning antimicrobial resistance in Nepal. All antibiotics are available over the counter (OTC) in Nepal without medical prescriptions; and this is a major challenge as it contributes to antibiotic pressure and development of resistance [39, 53]. The availability of OTC antibiotics and its consumption before arriving to hospitals may also confound the clinical presentation including general culture and sensitivity tests [39]. Thus, cautious evaluation of preceding treatment history, combined with strong suspicion for ESBL and MDR and its diagnosis may inform the appropriate treatment [7, 22]. The findings in this study warrant a relevant stakeholder's engagement to strengthen the health policy to rationalize the use of antibiotics including promoting diagnostic-based antibiotic prescriptions [54]. Specifically, in pediatric patients with urinary tract infections, it is critical to establish the diagnosis of ESBL organisms before initiating the antibiotic treatment.

Conclusion

High prevalence of multi-drug resistant ESBL organisms found in this study warrants restricting empirical treatment of the bacterial infection. The emergence of antibacterial resistance and production of ESBLs might be responsible for the frequently observed empirical therapy failures. Identification of ESBL producers in routine treatment of infectious diseases can reduce unnecessary and inappropriate antimicrobial use and can reduce the preventable morbidity and mortality. Imipenem, nitrofurantoin, piperacillin/ tazobactam and amikacin could be the drug of choice for the treatment of disease caused by ESBL producing bacteria.

Abbreviations

AMR: Antimicrobial Resistance; MDR: Multi-Drug Resistance/Resistant; ESBL: Extended Spectrum Beta-Lactamase(s); ATCC: American Type Culture Collection; CLSI: Clinical Laboratory Standard Institute; CFU: Colony Forming Unit(s); UTI: Urinary Tract Infection

Declarations

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Availability of data and materials

All data pertaining to this study are within the manuscript

Author's contributions

All the authors made substantial contribution to the study. KS and BD conceived and designed the study. KS collected samples, investigated and recorded the laboratory findings. KRR, SK and PG advised and formulated the methodology for the study. KRR and BA are responsible for reviewing several versions of manuscript. Others helped to review and amend this manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Institutional Review Committee of Nepal Health Research Council (NHRC) approved this research. Written consent was applicable to literate people while verbal consent was approached for the rest subjects. Parents/Guardians were interviewed in case of children. Strict adherence to the ethical guidelines was taken and we declare that this research is free from selection bias.

Consent for publication

Not applicable.

Competing interests

All the authors declared that they have no competing interests.

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Tables

Table 1: Distribution of bacterial isolates in various clinical specimens of children

Samples	Total (%)	Bacterial isolates		
		<i>E. coli</i> (%)	<i>K. pneumoniae</i> (%)	<i>K. oxytoca</i> (%)
Urine	87(84.5)	68(86.0)	14(77.8)	5(83.3)
Pus/ Wound pus	11(10.7)	7(8.8)	3(16.7)	1(16.7)
ET tube	2(1.9)	2(2.5)	0(0.0)	0(0.0)
Catheter tip	2(1.9)	1(1.3)	1(5.5)	0(0.0)
Blood	1(1)	1(1.3)	0(0.0)	0(0.0)
Total	103(100.0)	79(76.7)	18(17.5)	6(5.8)

Table 2: Antibiotic susceptibility pattern of bacterial isolates (*E. coli* and *Klebsiella* spp)

Antibiotics	Antibiotic Susceptibility pattern of <i>E. coli</i> and <i>Klebsiella</i> spp							
	<i>E. coli</i>		<i>K. pneumoniae</i>		<i>K. oxytoca</i>		Total	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Gentamicin	66 (83.5)	13(16.5)	12(66.7)	6(33.3)	6(100.0)	0(0.0)	84 (81.5)	19 (18.5)
Amikacin	64 (81.0)	15(19.0)	16(88.9)	2(11.1)	5(83.3)	1(16.7)	85(82.5)	18 (17.5)
Ciprofloxacin	40 (50.6)	39(49.4)	6(33.3)	12(66.7)	3(50.0)	3(50.0)	49 (47.5)	54 (52.5)
Ampicillin	22 (27.8)	57(72.2)	1(5.6)	17(94.4)	2(33.3)	4(66.7)	25 (24.3)	78 (75.7)
Piperacillin\	70	9(11.9)	15(83.3)	3(16.7)	6(100.0)	0(0.0)	91(88.3)	12 (11.7)
Tazobactam	(88.61)							
Imipenem	73 (92.4)	6(7.6)	14(77.8)	4(22.2)	6(100.0)	0(0.0)	93 (90.3)	10 (9.7)
Aztreonam	60 (76.0)	19(24.0)	11(61.1)	7(38.9)	5(83.3)	1(16.7)	76 (73.7)	27 (26.3)
Cefotaxime	35 (44.3)	44(55.7)	3(16.7)	15(83.3)	4(66.7)	2(33.3)	42 (40.8)	61 (59.2)
Ceftriaxone	37 (46.8)	42(53.2)	7(38.9)	11(61.1)	4(66.7)	2(33.3)	48 (46.6)	55 (53.4)
Ceftazidime	37(46.8)	42(53.2)	7(38.9)	11(61.1)	4(66.7)	2(33.3)	48 (46.6)	55 (53.4)
Cefepime	42(53.2)	37(46.8)	11(61.1)	7(38.9)	4(66.7)	2(33.3)	57(55.4)	46 (44.6)
Cefoxitin	53(67.1)	26(32.9)	7(38.9)	11(61.1)	5(83.3)	1(16.7)	65 (63.1)	38 (36.9)
Nitrofurantoin	72(91.2)	7(8.8)	12(66.7)	6(33.3)	4(66.7)	2(33.3)	88 (85.4)	15 (14.5)

Table 3: ESBL production profile among *E. coli* and *Klebsiella* species

Organisms	No. of isolates	ESBL producer	
		Suspected (%)	Confirmed (%)
<i>E. coli</i>	79	42(53.2)	22(27.8)
<i>K. pneumoniae</i>	18	9(50)	6(33.3)
<i>K. oxytoca</i>	6	2(33.3)	1(16.7)
Total	103	53(51.5)	29(28.2)

Table 4: Distribution of ESBL according to gender and age of children

Age groups	No. of isolates (%)	ESBL production	p-value
≤5 years	80(77.7)	24(82.8)	0.837*
6-10 years	16(15.5)	4(13.8)	
11-15 years	7(6.8)	1(3.4)	
Total	103(100.0)	29(28.1)	

* Chi-square test

Table 5: Distribution of ESBL producers in different wards of hospitals

	No. of isolates (%)	ESBL producer bacterial isolates (%)	p-value	
Wards				
Out-patients				
OPD	52(50.5)	14 (48.3)	0.737*	
In- patients				
ICU	13(12.6)	5 (17.2)		
Other wards	38(36.9)	10 (34.5)		
Total	103(100.0)	29 (28.1)		

* Chi-square test

Figures

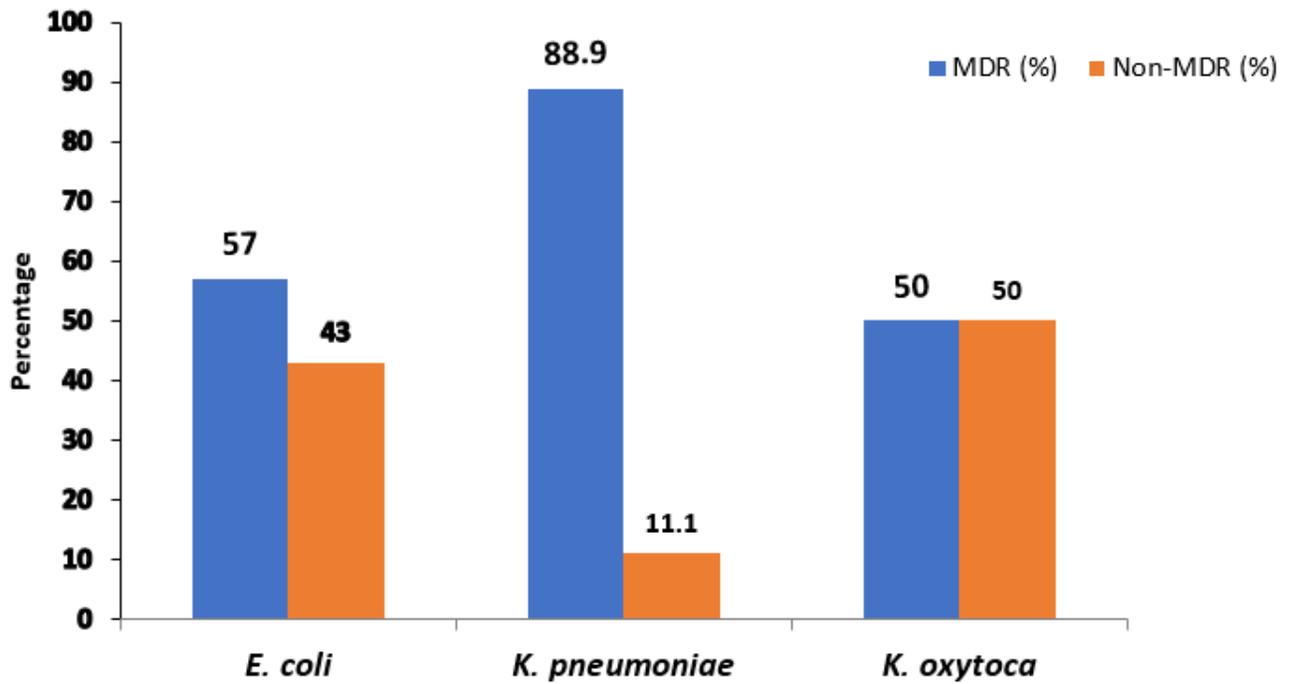


Figure 1

MDR profile in bacterial isolates

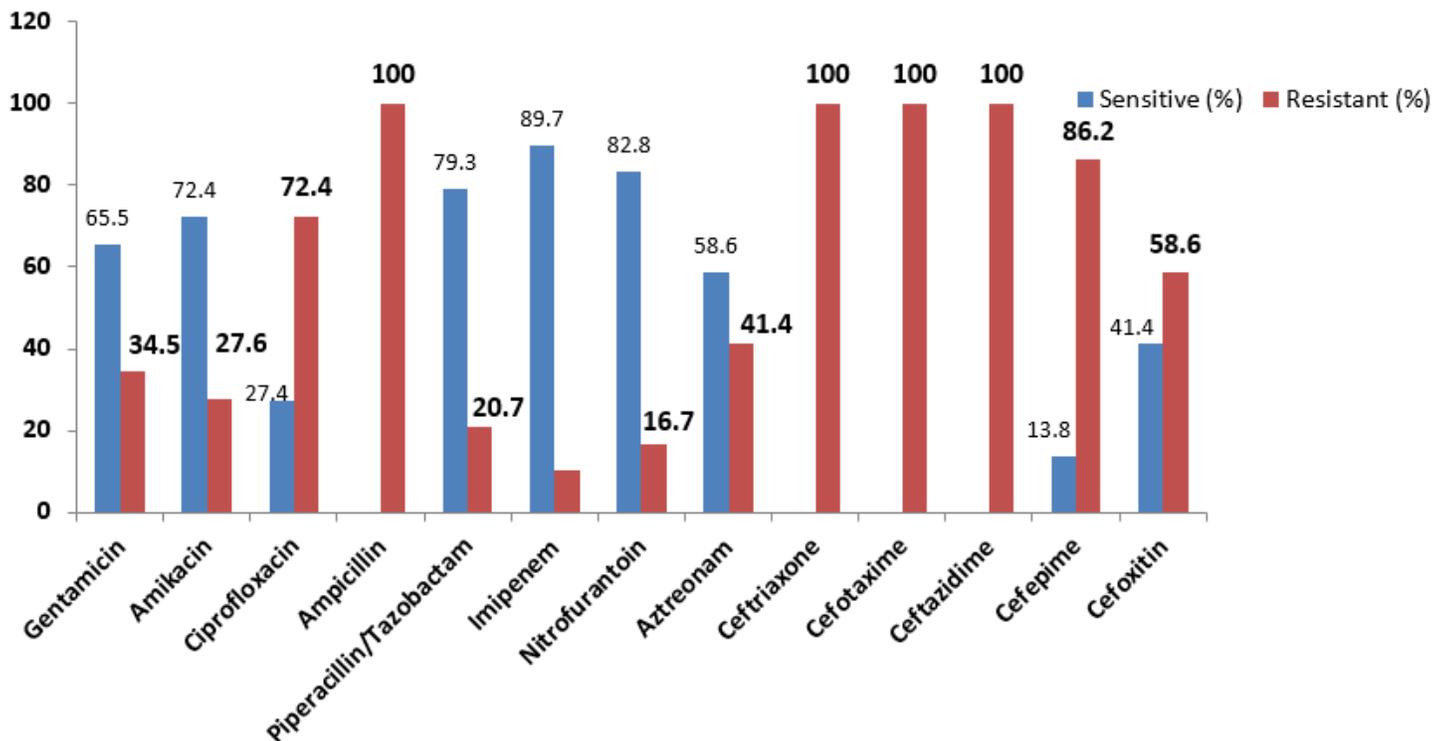


Figure 2

Antibiotic susceptibility pattern of ESBL producers