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Screening of ESBL Producing Multidrug Resistant *E. coli* from Urinary Tract Infection Suspected Cases in Southern Terai of Nepal

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Abstract

Background and objectives: Multidrug-resistant *E. coli* expressing extended-spectrum β-lactamase pose serious challenges to clinicians for the therapeutic management of clinical cases in urinary tract infection. The ability of beta-lactamase to cause resistance varies with its activity, quantity, cellular location of gram-negative organisms and its permeability of the producer strain. Therefore, this study was focused to determine the dominance of MDR *E. coli* and evaluation of status of β-lactamase enzyme produced by MDR *E. coli*.

Materials and methods: A total of 321 successive midstream urine samples were processed among suspected cases of urinary tract infection. Standard microbiological techniques were used for isolation and identification of uropathogens. The antimicrobial susceptibility pattern of bacterial isolates was determined by Kirby-Bauer Disc Diffusion technique. The MDR *E. coli* isolates were screened for ESBL by double disc synergy test and confirmed with combined disc diffusion test. The p- value<0.05 was considered as statistically significant.

Results: The maximum numbers of MDR *E. coli* isolates were isolated as of female patients with 55.75% than male of 44.24%. Most of the MDR *E. coli* isolates were isolated from less than 20 years with 30.97% and was dropped in between 40-60 years with 20.35%. The MDR *E. coli* isolates in association with gender and age group was found to be statistically insignificant (p=0.310). Among 69 suspected ESBL *E. coli* isolates, 62.31% were confirmed as ESBL producer.

Conclusion: *E. coli* isolates showed shocking rate of drug resistance to the frequently prescribed drugs. The high rate of ESBL-producing MDR *E. coli* was also observed. There is an increasing need for periodic monitoring of drug susceptibility pattern to prevent the spread and development of antimicrobial resistant strains and ESBL producers.

Keywords: *Escherichia coli*; Extended spectrum β-lactamase; Multidrug resistance; Urinary tract infection

Introduction

Urinary Tract Infection (UTI) is the commonest bacterial infection prevalent to both female and male. It is expected that about 35% of healthy women experiences warning signs of UTIs [1]. The incidence is more frequent in women than men due to squatness of female urethra, dearth of prostatic secretions, easy contamination with fecal flora and pregnancy [2]. The dominance of this disease is additional in developing countries owed to deprived sanitation, living method, undernourishment, and ecological stipulation [3]. Mostly, neonates, girls, young women, infants, young children and older men are mainly vulnerable to UTIs [4].

UTIs involves bacterial invasion and multiplication of the pathogen in the organs of the urinary tract system is classified into uncomplicated and complicated infections on the basis of organ involved [5,6]. Infection may be expressed predominantly as pyelonephritis, pyelitis, ureteritis, cystitis, prostitis and urethritis but the entire urinary tract is always at risk of invasion by bacteria [3]. Microorganisms belonging to Enterobacteriaceae have been documented as elementary reason of nosocomial and community acquired UTIs [7]. The infinite majority of uncomplicated UTIs are caused by the Gram negative bacilli and with other pathogens including *Enterococci, Staphylococcus saprophyticus*, *Klebsiella* spp, *Pseudomonas* spp, *Proteus* spp, *Staphylococcus aureus* and *Proteus mirabilis* [4].

Numerous studies have barbed towards high incidence rate of UTI associated with *E. coli* and antibiotic resistance. The emergence of Multi Drug Resistant (MDR) variant of *E. coli* has been accounted [8,9]. MDR is defined as resistance to at least two antibiotics of different classes including aminoglycosides, chloramphenicol, tetracyclines and/or erythromycin [10,11]. MDR in many bacteria is due to the action of multi-drug efflux pumps and by the accumulation on Resistance (R) plasmids or transposons, of genes with each coding for resistance to a specific agent [10,12]. Nowadays, in UTIs Extended-Spectrum Beta-Lactamase-expressing Gram-Negative Bacilli (ESBL-GNB) generally cause community-acquired infections [13]. The resistance of Gram-negative bacteria is typically owed to plasmid-mediated enzymes called Extended-Spectrum B-Lactamases (ESBLs) [14]. ESBL producing bacteria are typically associated with MDR and antibacterial choice is often complicated by multi-drug resistance [15-17].

Extended-spectrum β-lactamase and metallo β-lactamase producing bacteria are up-and-coming apprehension for health professionals. Patients with increased threat of increasing colonization or infection with ESBL producing microorganisms are repeatedly fatally sick patients with prolonged hospital stays [18]. They are frequently resistant to numerous antibiotic classes, including fluoroquinolones and aminoglycosides. Resistance to β-lactam antibiotics has increased significantly in the last two decades and has been documented in both community and hospital settings [19-21]. Current updated knowledge of the susceptibility pattern of bacteria is vital for the appropriate assortment and utilization of antimicrobial drugs and, also for the succession of suitable prescribing guidelines [22]. The infections caused by MDR pathogens, the rate of emergence and spread of antibiotic resistance cannot be reduced without gathering information about the existing MDR strains [23]. Although, ESBL and MBL have been studied well in Nepalese community [24,25] but scanty information has been witnessed related to multi drug resistant variant of *E. coli* reported from the Southern Terai of Nepal. Therefore, this study was designed to isolate and identify the causative agent of UTI, determination of prevalence of MDR among the pathogens and to evaluate the status of β-lactamase enzyme producer MDR *E. coli*.

Materials and Methods

Study design

The present research work was a hospital based cross-sectional study which was conducted in the Microbiology Unit of Clinical Pathology Laboratory at Janaki Medical College Teaching Hospital (JMCTH), Janakpur, Nepal from April to December, 2014.

Ethical consideration

Informed consent was attained from the participant and work approval was taken from the institutional ethical committee of JMCTH, Janakpur, Nepal earlier to the study. A term paper of information letter and consent form was prearranged to patients ahead of participating in the research. In case of ignorant and uneducated participants, information was endowed with interpretation of the consent form in presence of eyewitness in the local language. The information of patients such as name, sex, age and clinical history were collected. If the patients were children, the information was gathered related to the subject matter with the help of their parents.

Inclusion and exclusion criteria

The patient's history with complaint of nausea, micturition, dysuria, polyuria, haematuria, suprapubic tenderness, pain or pressure in back or lower abdomen was included. Those clinical samples which showed poly-microbial and insignificant growth, incomplete culture form, without proper labeling including date, time, age, lab number and sex were excluded.

Specimen collection

The Mid Stream Urine (MSU) samples (10-20 ml) were collected in the sterile clean dry wide mouthed leak-proof bottle. The idiosyncratic instruction was followed by the patient for the sample collection. The container was then labeled properly with serial no., age and sex. When instantaneous processing was not achievable, the sample was frozen at 4-6°C, and likely boric acid (1.8% w/v) was added as preservative to the urine with delay of more than 2 h estimated [26].

Culture of specimen

Medias were set as instructed by the manufacturer company (Himedia). The urine specimen was streaked on the MacConkey Agar (MA) and Blood Agar (BA) medium by the semi-quantitative culture procedure *via* a standard loop. After mixing the urine sample in the container methodically, a loopful of sample was contacted to the centre of the plate, from which the inoculum was spread in a line across the diameter of the plate. The loop was drawn across the entire plate devoid of flaming crossing the first inoculum streak abundantly to produce isolated colonies. The plates were incubated aerobically at 37°C overnight. The fairly accurate numeral of colonies was count up. The number of bacteria i.e. Colony Forming Unit (CFU) per ml urine anticipated in accordance to the volume of urine inoculated formerly and testimonized as-Less than 10^4/ml organisms: not significant, 10^4-10^5/ml organisms: doubtful significance (suggest repeat specimen) and more than 10^5/ml organisms: Significant bacteriuria.

Identification of the isolates

Detection of significant bacterial isolates were done by using microbiological techniques as illustrated in the Bergy's manual which entails morphological appearance of the colonies, staining reactions and biochemical properties.

Antimicrobial susceptibility testing (AST)

Antibiotic sensitivity test for the isolated organism were done by using Kirby Bauer Disc Diffusion technique following the definition of the National Committee of Clinical Laboratory Standards [27]. Bacterial inoculums were prepared by suspending the freshly-grown bacteria in 25 ml sterile Nutrient broth. A sterile cotton swab was used to streak the surface of Mueller Hinton agar plates. Filter paper disks restraining designated amounts of the antimicrobial drugs were acquired from commercial supply firms (Himedia Labs, Mumbai, India). Interpretation as 'Sensitive' or 'Resistant' was completed on the base of the diameters of zones of inhibition of bacterial growth as suggested by the disc manufacturer.

After performing the antimicrobial susceptibility testing, MDR isolates in pure culture were preserved in 20% glycerol containing Tryptic soya broth and kept at -70°C until subsequent tests for the existence of ESBL was completed.

Screening and confirmation for ESBL producers

The MDR *E. coli* isolates were screened for possible ESBL producer using ceftazidime (30 μg) and cefotaxime (30 μg) [27]. According to the guiding principle, isolates showing ceftazidime<22 mm and cefotaxime<27 mm are the potential ESBL producing strains. The screen positive isolates i.e. suspected ESBL producers were subjected to Combined Disk (CD) test for authentication of ESBL production using MASTDISCSTM Extended Spectrum β-Lactamase (ESBL) detection discs. The kit consisted of:

Set 1: Ceftazidime (30 μg) and ceftazidime (30 μg) plus clavulanic acid (10 μg)

Set 2: Cefotaxime (30 μg) and cefotaxime (30 μg) plus clavulanic acid (10 μg)

Quality control

Laboratory tools and equipment like refrigerator, incubator, autoclave and hot air oven were routinely monitored for their effectiveness. The temperature of refrigerator and incubator was observed on a daily basis for their performance and instantly corrected if any variations found. Reagents and media were frequently monitored for their manufacture, expiry date and proper storage. After preparation, they were properly labeled with preparation date. The quality of media prepared was ensure by incubating one plate of each lot for sterility and using standard control strains for performance testing. During identification of organisms, for each test *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains for culture and sensitivity testing. Strict aseptic conditions were kept up while carrying out all the procedures.

Statistical analysis

The analysis of data was done by using SPSS 20.0 version and Microsoft Excels 2007. The p-value<0.05 was considered statistically significant.

Results

Prevalence of UTI among suspected respondents

A total number of patients attending OPD, JMCTH were 321 of which 150 were male and 171 as female. Among them, 202 (62.92%) had UTI in which 48.01% were male and 51.98% female and rest of them were free of UTI. The prevalence of UTI in relation to gender was found to be statistically insignificant (p=0.985). The outcomes are shown in Table 1.

Gender	UTI		Total OPD patients	Statistics
	Positive	Negative		
	No. (%)	No. (%)		
Male	97 (48.01)	53 (44.53)	150	χ^2=0.364 p=0.985
Female	105 (51.98)	66 (55.46)	171	
Total	202 (62.92)	119 (37.07)	321	

Table 1: Microbial growth among the total suspected patients.

Age group (yrs)	Gender		Total bacterial growth (%)	Statistics
	Male (%)	Female (%)		
<20	19 (19.58)	22 (20.95)	41 (20.29)	χ^2=1.152 p=0.997
20-40	46 (43.80)	47 (48.45)	93 (46.03)	
40-60	27 (27.83)	29 (27.61)	56 (27.72)	
>60	4 (4.12)	8 (7.61)	12 (5.94)	
Total	97 (48.01)	105 (51.98)	202	

Table 2: Bacterial growth among positive UTI respondents.

Antibiotic used	Sensitive	Intermediate	Resistant
	No. (%)	No. (%)	No. (%)
Imipenem	77 (62.60)	20 (16.26)	26 (21.13)
Doxicycline	39 (31.70)	33 (26.82)	51 (41.46)
Norfloxacin	29 (23.57)	13 (10.56)	81 (65.85)
Cephalexin	9 (7.31)	17 (13.82)	97 (78.86)
Nitrofurantoin	54 (43.90)	4 (3.25)	64 (52.03)
Ciprofloxacin	29 (23.57)	22 (17.88)	72 (58.53)
Cotrimoxazole	21 (17.07)	14 (11.38)	88 (71.54)
Amikacin	75 (60.97)	28 (22.76)	20 (16.26)
Ofloxacin	31 (25.20)	13 (10.56)	79 (64.22)
Nalidixic Acid	6 (4.87)	26 (21.13)	91 (73.98)
Ceftazidime	32 (26.01)	13 (10.56)	78 (63.41)
Ceftriaxone	29 (23.57)	11 (8.94)	83 (67.47)

Table 3: Antibiotic susceptibility pattern of *E. coli*.

Age and gender wise distribution of significant bacterial growth

Among total positive UTI respondents, the significant growth of bacterial isolates were observed more in between 20-40 years age of 46.03% followed between 40-60 years (27.72%) and less than 20 years (20.29%). Visible bacterial growth was found lesser in above 60 years. In almost all age groups, female's urine specimen showed highest bacterial growth than male. The relation between the age and gender wise pattern of bacterial isolates were found statistically insignificant (p=0.997) as shown in Table 2.

Microbial profile of urinary isolates isolated from respondents

A total of 202 bacterial isolates were identified in which 123 isolates were *E. coli*, 27 were *K. pneumoniae*, 3 were *P. aeruginosa*, 38 were *P. vulgaris*, 4 were *S. aureus* and 7 were *C. diversus*. Among all bacterial isolates, *E. coli* was found to be major micro-organism to cause UTI. The results are revealed in Figure 1.

Antibiotic susceptibility pattern of *E. coli*

Of 12 different antibiotics used against 123 *E. coli* isolates, imipenem was found to be the drug of choice with the susceptibility of 62.60% chased by amikacin with 60.97% and nitrofurantoin with 43.90% but cephalexin and nalidixic acid was observed least effective drug which showed the highest resistance with 78.86% and 73.98% respectively. The results are depicted in Table 3.

Age and gender wise distribution of MDR *E. coli*

Among 123 *E. coli* isolates, 113 were MDR *E. coli* strains. The highest figures of bacterial isolates were found in female patients with 55.75% than male of 44.24%. Most of the MDR *E. coli* strain was isolated from less than 20 years with 30.97% and drop was observed in

between 40-60 years with 20.35%. The MDR *E. coli* isolates in association with gender and age group was found to be statistically insignificant (p=0.310). The results are as given in Table 4.

ESBL production of MDR *E. coli*

A total of 123 *E. coli* isolates were isolated, of which 113 were MDR strains where 69 isolates were suspected as ESBL producers. Among suspected cases, the total numbers of confirmed cases were 43 (62.31%) as shown in Table 5.

Screening for ESBL production of MDR *E. coli*

Of the total 113 MDR *E. coli* isolates, 69 MDR *E. coli* isolates suspected as producers of β-lactamases were screened for ESBL production using ceftazidime and ceftriaxone as the CLSI suggested screening agents. The sensitivity and positive prediction value of Ceftazidime was more than Cefotaxime. There were significant association between ESBL production with ceftazidime and cefotaxime as shown in Table 6.

Patterns of ESBL production confirmed by combination disks assay

Of 43 screened ESBL *E. coli* isolates were subjected for ESBL confirmation test using different combination disks with ceftazidime-clavulanate and cefotaxime-clavulanate in which all the screened cases were established as ESBL positive as shown in Table 7.

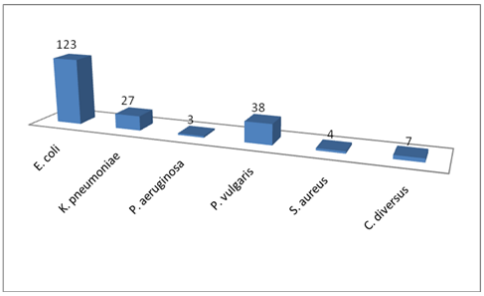

Figure 1: Frequency pattern of total microbial isolates.

Age groups (years)	Gender		Total MDR *E. coli*	Statistics
	Male (%)	Female (%)		
< 20	13 (26)	22 (34.92)	35 (30.97)	χ^2=9.387 p=0.310
20-40	8 (16)	19 (30.15)	27 (23.89)	
40-60	10 (20)	13 (20.63)	23 (20.35)	
>60	19 (38)	9 (14.28)	28 (24.77)	
Total	50 (44.24)	63 (55.75)	113	

Table 4: Pattern of MDR *E. coli* isolated from total respondents.

Organism	Total isolates (%)	No. of MDR Strains (%)	No. of suspected ESBL production (%)	No. of cases confirmed (%)	Negative cases on confirmation (%)
E. coli	123 (38.31)	113 (91.86)	69 (61.06)	43 (62.31)	26 (37.68)

Table 5: MDR *E. coli* produces β- lactamases.

Screening agent	ESBL screening of suspected cases		No. of confirmed ESBL producer	Sensitive (%)	Positive predictive value (%)	Statistics
Ceftazidime (30 µg)	Screen positive	44	38	88.37	86.36	χ^2=29.89 p=0.000
	Screen negative	25	5			
Cefotaxime (30 µg)	Screen positive	49	36	83.72	73.46	χ^2=7.70 p=0.005
	Screen negative	20	7			

Table 6: Screening for ESBL production of MDR *E. coli*.

Combination disks assay	Criteria for confirmation	No. of suspected ESBL	No. of confirmed cases	Total confirmed cases	Negative cases after confirmation
CAZ (30 µg) and CAZ (30 µg) plus CV (10 µg)	Increase zone size of >5 mm with >1 of the combination disks	69	43	43	26
CAZ (30 µg) and CTX (30 µg) plus CV (10 µg)			43		

Table 7: Confirmation of ESBL by combination disks.

Discussion

UTIs are generally widespread nosocomial infection in Nepal [28]. UTI is associated with alterations in the host's systemic or local immunity, such as immune-suppression, diabetes, hyper-adrenocorticism, anatomic abnormalities (polyps/tumors, recessed vulva), indwelling catheters, uroliths, or urethral sphincter mechanism incompetence. *E. coli* are considered as a normal component of gastrointestinal and distal urogenital flora, but it can ascend the urethra and gain entrance to the urinary tract. Specific virulence factors found in *E. coli* allow it to adhere to and invade host cells, produce toxins, utilize host nutrients, and evade the host's immune system [29,30].

The present study enrolled 321 suspected UTI patients where 62.92% suffered from UTIs in which 48.01% were male and 51.98% female. Similar type of study conducted by Chaudhari et al. found 25.60% were male and 74.40% were female [31]. Both study showed the higher prevalence of UTI take place in female. The probable reasons for UTI in young women may be due to short urethra and its opening, complex physiology especially during gestational period. Additionally, certain variety of contraceptives can also encourage the peril of UTIs [4,32].

The present study depicts that the age grouping of 20-40 years had the highest visible bacterial growth and least growth was observed above 60 years. Amongst the isolates, the highest integer of pathogens was isolated from sample of female patients (51.98%) as compared to male (48.01%). Similar result was reported in the study conducted at National Public Health Laboratory, Teku, Kathmandu, Nepal by Thakur et al. [33]. These parallel results are in concord with earlier studies [32,34-36]. This may be due to the incidence of UTI increases with age and sexual activity. Vast majority of acute symptomatic infections involve young women. But, in contrast Thakur et al. noted high prevalence of UTI in old age in male subjects [33] which may be exceptional to diverse circumstances like prostatitis, diabetes, pathetic immune status and past antibiotic treatments in associated diseases.

This study reports 202 bacterial isolates were identified in which 123 isolates were *E. coli*, 27 were *K. pneumoniae*, 3 were *P. aeruginosa*, 38 were *P. vulgaris*, 4 were *S. aureus* and 7 were *C. diversus*. Among all bacterial isolates, *E. coli* was found to be predominant organism to cause UTI. The study accomplished at Microbiology Department of National College, Kathmandu, Nepal by Yadav et al. observed that of 25 samples showing significant bacteriuria were caused by *E. coli* (84%) whereas *Citrobacter diversus*, *Klebsiella pneumoniae* and *Staphylococcus aureus* with 16% [4]. Nalini and Sumathi found 356 *E. coli*, 123 *Pseudomonas* spp, 73 *Klebsiella* spp and 54 *Proteus* spp were identified in their study [37]. Similarly, Wadekar et al. reported *E. coli* (67.7%) was the most universal isolate followed by *Staphylococcus* and *Klebsiella* spp [38]. Out of 367 urine samples processed in the study organized by Kulkarni et al. yielded 96 were *E. coli* and 58 were *Klebsiella* species [39]. Chaudhari et al. isolated bacterial pathogen from UTIs as *E. coli* (66%), *Klebsiella* spp. (12%), *Enterococcus* spp. (8%), *Pseudomonas* spp. (6%), *Acinetobacter anitratus* (5%), *and Proteus* spp. (3%) [31].

Likewise, a study conducted by Yadav and Prakash in 2015 at Microbiology Unit, Clinical pathology laboratory in the collaboration with Medicine department at Janaki Medical College Teaching Hospital, Janakpur, Nepal also noted the highest number of *E. coli* isolates involved in UTI among diabetics [32]. Shrestha et al. reported in 2016, *E. coli* (71.3%), *K. pneumoniae* (9.8%), *K. oxytoca* (8.6%), *Proteus* spp. (4.6%), *C. fruendi* (2.3%), *Pseudomonas* spp. (1.7%), *Enterobacter* spp. (1.1%) and *Acinetobacter* spp. (0.6%) isolates from UTIs [7]. Chaudhary et al. found in their study 67% and 18% of infection was produced by *E. coli* and *Staphylococcal* species respectively and others were *Klebsiella*, *Citrobacter* and *Pseudomonas* spp [40]. Mishra et al. reported *E. faecalis*, *S. aureus*, *C. freundii*, *E. aerogenes*, *E. coli*, *K. oxytoca*, *K. pneumoniae*, *P. vulgaris* and *P. aeruginosa* were isolated from UTI patients [41]. Parajuli et al. accounted *E. coli* (68.4%) leading pathogen involved in UTI [42].

From all the above study, *E. coli* was the major pathogen concerned with UTI. As *E. coli* is a common pathogen which is usually a commensal bacterium of humans. Intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infection, meningitis, peritonitis, and septicemia are caused by pathogenic variants [43]. *E. coli* have unique virulent properties that can bind to Gal alpha1-4 Gal receptor of the uroepithelial cells of human urinary tract which can commence infectivity itself and contributory to a foremost uropathogen throughout the world [44]. The attachment of *Proteus* species to uroepithelial cells instigate the secretion of interleukin 6 and interleukin 8 in the mucosal endothelial cells and induces apoptosis and epithelial cell desquamation. Urease production, in concert with the attendance of bacterial motility and fimbriae, may errand the fabrication of upper urinary tract infections by *Proteus* spp. [45]. Patients who are on ventilators, catheters or surgery wounds are highly prone to catching Hospital-acquired infection by *Klebsiella* spp. [46].

Antimicrobial resistance is now recognized as an increasingly global problem, especially in Gram-negative bacteria [47]. UTIs are mainly treated with β-lactam antibiotics. However, acquired resistance to these antibiotics in UTI pathogens is commonly augmented by bacterial enzymes, and leads to the emergence of ESBLs [48]. In this study, 12 different antibiotics used against 123 *E. coli* isolates in which cephalexin, nalidixic acid, cotrimoxazole, ceftriaxone and ceftazidime showed the highest resistivity with 78.86%, 73.98%, 71.54%, 67.47% and 63.41% respectively. But, the study conducted by Perez et al. accounted 94% *E. coli* isolates to be resistant to ceftriaxone [49]. This high rate of resistance may be due to the lack of antibiotic policy and the irrational use of third generation cephalosporins, mainly ceftriaxone in the hospital [50]. *E. coli* was highly resistant to ciprofloxacin (81%) which is analogous with this study conducted by Haque and Salam [51].

Increasing resistance to broad spectrum cephalosporins amongst *E. coli* and *Klebsiella* species predominantly due to the production of ESBLs were accounted from several countries [52,53]. The established fact is that the increased level of drug resistance seen among *E. coli* is mediated by β-lactamases, which hydrolyze the β-lactam ring inactivating the antibiotic, the classical TEM-1, TEM-2, and SHV-1 enzymes are the predominant plasmid-mediated β-lactamases of gram-negative rods [10,48]. Furthermore, self-medication practice, easy availability of drugs from pharmacy, its use without doctor's prescription and loopholes in guidelines in drug policy in developing countries like Nepal might be a prime cause of antimicrobial resistance.

This study signifies that the antibiotic susceptibility pattern of *E. coli* showed that imipenem, amikacin and nitrofurantoin was the most effective drug. Also, similar results had seen documented in previous studies [31,33] which are in accordance with this study. Amikacin was developed from kanamycin to chunk the access of a diversity of kanamycin-modifying enzymes to their target sites [54]. Among the

various aminoglycoside-modifying enzymes, Acetyltransferases (AAC (6')-I and AAC (6')-APH (2')), Adenyltransferases (ANT (4')-I and ANT (4')-II), and Phosphotransferases (APH (3')-II and APH (3')-III) have been revealed to upshot in the modification of amikacin [55] which demonstrates a reasonably high prevalence of amikacin susceptibility which is typically pragmatic among members of the family Enterobacteriaceae. The advanced vulnerability of *E. coli* to nitrofurantoin may be prejudiced by nitrofurantoin's narrow spectrum of activity, inadequate indication, narrow tissue distribution and limited contact with bacteria outside the urinary tract [56].

This study highlights that *E. coli* showed the predominant number of MDR strains (91.86%). Higher rate of MDR was found in female patients (55.75%) compared to male (44.24%). The outcomes of this study are similar to Thakur et al. [33].

Problems in UTIs have been augmented because of the occurrence of ESBL producing *E. coli* due to overuse of third generation cephalosporins and monobactams [57]. The present study noted 69 MDR *E. coli* isolates that were suspected of being producers of β-lactamases were screened for ESBL. Among suspected cases, 62.31% were found as confirmed cases. Shrestha reported high prevalence of MDR *E. coli* (65.0%) isolates were ESBL positive [58]. This rate is similar to additional studies that accounted as 40-70% [33,39,59-70]. Kashyap et al. in 2013 reported the prevalence of ESBLs production was 37% [71] which correlates with Kumar et al. in 2006 and Jalalpour in 2011 [72,73]. But, the study conducted by Hazir et al. noted only 23.56% ESBL prevalence in *E. coli* which is analogous result reported by another study [74,75]. This is not in accord with the present study because of disparity in geographical variation and study design.

Conclusion

The present study highlights the therapeutic future of the β-lactam antibiotics is serious dilemma and gigantic challenge to clinicians. Nowadays, UTIs caused by ESBL-producing *E. coli* has emerged more rigorously. Therefore, early identification of infections due to this pathogen is essential for rapid establishment of appropriate treatment and to reduce the mortality in hospital and community setting. In order to prevent and control the emergence of antimicrobial resistance in ESBL producing organisms, it is of utmost importance to edge the misuse and overuse of antibiotics especially broad spectrum antibiotics. Further, the recognition of antimicrobial resistance and more specifically towards β-lactam drugs should be routinely surveyed and monitored at different time interval. These findings also suggest integrating early and sensitive methods to detect ESBL producing strains should be practiced so that appropriate antibiotics can be used. It is also important to formulate appropriate community as well as hospital antibiotic policies to decrease the spread of ESBL producing microorganisms. Moreover, researches are desirable for finding novel drugs and their rational use in coming future.

Limitation

The study was carried out in only one hospital of this area which does not reveal the cyclic, geological and tribal difference of pathogens and their antibiotic susceptibility profile picture of the whole country. The genotype of ESBLs among *E. coli* isolates was not detected due to lack of feasibility of time duration and resources available in the laboratory.

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Author's Contribution

KY-Concept and design of study, inscription of manuscript and descriptive data analysis, and final approval of manuscript. **SP**-Reviewed literatures and involved in scripting first draft of manuscript, revision of final manuscript with critical intellectual content.

Source of Support

All the logistic supports were provided by Janaki Medical College teaching Hospital, Janakpur, Nepal.

Conflict of Interest

None declared.

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